SPINAL CORD NOCIRECEPTIVE NEURONES

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C O N T E N T S

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SUMMARY

These electrophysiological investigations of spinal cord neurones responding to noxious and non-noxious cutaneous stimulation were conducted to evaluate (1) their ascending projection into the spinothalamic tract, (2) the involvement of endogenous opioids in mediating the tonic descending and segmental inhibition and (3) the role of the nucleus locus coeruleus (LC) in modulating spinal cord nociceptive transmission.

A total of 252 neurones were antidromically activated from the ventrolateral quadrant but only 14 of these were found to project into the spinothalamic tract. The spinothalamic tract (STT) neurones responded to noxious and non-noxious, or to noxious stimulation alone. All the units were located in lamina VII and most of them displayed wide receptive fields. None of the 39 specific nociceptor-driven neurones from lamina I was found to project into the spinothalamic tract.

Naloxone (0.3-2.0 mgm/kg) did not alter the response of multireceptive neurones to heat, to tonic descending inhibition or to the inhibition generated by stimulation of contralateral plantar nerve or dorsal columns.

Stimulation in the nucleus locus coeruleus produced a predominantly inhibitory effect on nociceptive transmission in the spinal cord. The descending inhibition produced from LC was antagonised by the

administration of α-noradrenergic receptor antagonists but was not changed by β-receptor antagonists. Methysergide did not alter the inhibition from LC or NRM whereas the GABA antagonist, bicuculline, abolished the inhibition. Naloxone partially reduced the inhibition from LC. The ipsilateral ventral quadrant lesion (VLF and VF) abolished the actions produced from LC but a bilateral DLF lesion was required for abolishing the NRM actions. Electrolytic lesions made in the midline raphe complex did not block the actions produced from LC. The stimuli in LC and NRM that evoked inhibition of multireceptive neurones also produced DRPs.

These data support the conclusion that the spinothalamic tract in the cat plays a role in the transmission of nociceptive and tactile messages to the brain. The endogenous opioids do not appear to be involved in mediating the types of inhibition examined (Section III). The descending actions produced from LC are not mediated through NRM and are most probably mediated through the direct coeruleospinal projection. Catecholaminergic, opioid and Gabaergic transmission is involved in mediating these actions which may involve both the pre- and postsynaptic mechanisms.

SECTION I

LITERATURE SURVEY

The main theme of the investigation on the spinal cord neurones that receive noxious and non-noxious cutaneous input was this: their ascending projection as well as segmental and descending control from the brian-stem.

The literature review that follows will focus on the central theme as well as various other aspects related to the main problem under investigation. A brief historical introduction of different theories of sensation, in particular, the sensation of pain will be discussed. This will be followed by a systematic account of the previous literature starting from cutaneous receptors and afferents.

Descartes in the middle of the seventeenth century provided the first classical description of the traditional theory of pain, the specificity theory, based on the plausible assumption of the existence of a specific pain system transmitting messages from pain receptors to a pain centre in the brain involved in the perceptual mechanisms. Later in 1842, Müller proposed an association of the quality of experience with the properties of sensory nerves, known as Müller's doctrine of specific nerve energies. The next important step was taken by Von Frey (1895) who proposed the role of cutaneous pain to free nerve endings and extended Müller's erroneous concept of a unitary sensory system underlying the somaesthetic sensation to four major cutaneous modalities (touch, warmth, cold and pain), each having a specific receptor structure. The major strength of Von Frey's theory lies in the concept of physiological specialisation whereas its weakness lies in the anatomical correlations.

Following the specificity theory, a search was made for the spinal pain pathway, based mainly on clinical evidence. Antero-lateral quadrant (ALQ) was suggested to be important for pain and as a consequence the spinothalamic tract which courses through ALQ came to be known as a 'pain' pathway. Based on the studies of Head and Sherren (1905) it was proposed that two parallel cutaneous systems exist, one subserving touch (epicritic) and the other subserving pain and temperature (protopathic) and that the medial lemniscus and ALQ pathways mediate the epicritic and protopathic sensations respectively. However even the bilateral dorsal column lesions preserve touch sensitivity thus indicating the role of other pathways. The specificity theory has been criticised by several workers (Head, 1920; Sinclair, 1955; Melzack and Wall, 1965) and it is the inherent psychological assumption, that a single stimulus dimension excites a specific receptor in the skin giving rise to a specific sensation, which is disputed. The main argument seems to be against the psychological specificity and not against the physiological specialisation, which is a fact. Because of the weakness of Von Frey's theory of specificity which lies in its erroneous assumption of psychological specificity, Goldscheider's (1894) pattern theory has survived. This is based on the proposal that pain is felt when the summation of inputs reach a critical level, with the central systems playing an important part in these summations (Livingston, 1943; Gerrard, 1951). Peripheral summation was also recognised as being important for the central interpretation of pain (Weddell, 1955; Sinclair, 1955).

Melzack and Wall (1965) proposed the 'Gate Control Theory' which recognised sensory information and motivational-affective components

as inherent for pain experience and considered these two processes to be simultaneous and not successive. A spinal cord gating mechanism in the substantia gelatinosa was proposed which could modulate the afferent inflow to the CNS before it evoked the pain sensation. The modulation of transmission would be dependent on the relative activity in the large versus the small fibres, the former enhancing the inhibitory effect of SG on the afferent input and the latter diminishing it and also on the descending influences from the brain. However there are several objections to the hypothesis. Zimmermann (1968) did not find any presynaptic hyperpolarisation caused by Aδ and C-fibres as would be predicted from this hypothesis, instead it was reported that a pure volley from C-fibres produced a negative dorsal root potential thus providing an indication of presynaptic inhibition. Similarly Franz and Iggo (1968) did not find positive dorsal root potentials on stimulation of C-fibres. Some investigators have reported the generation of positive dorsal root potential by volleys in fine afferents (Dawson et al. 1970). The assumption of inhibition of nociceptive input by activity in large fibres from mechanoreceptors was subsequently validated (Handwerker et al. 1975). After the proposal of this hypothesis, specific nociceptor driven neurones in lamina I projecting to the higher centres of the nervous system were reported, which may have an important part to play in nociception. The finding of specific nociceptor driven neurones, together with the present studies on the substantia gelatinosa(SG), showing it to be a heterogenous entity containing different types of neurone, will shed new light and lead to new ideas, as will be shown in the literature that follows.

CUTANEOUS INNERVATION

The skin is richly supplied by fibres both myelinated and non-myelinated, their diameters ranging from 14 μm to <1 μm. Group II fibres innervate corpuscular endings or hair follicle receptors whereas Group III (Aδ) thinly myelinated fibres supply hair follicle receptors and mechanical nociceptors (Iggo, 1974, 1977). C-fibres innervate mechanical, thermal and polymodal nociceptors as well as sensitive mechanoreceptors (hair), warm receptors and are also present in sympathetic efferents (Iggo, 1974; Zimmermann, 1979). Cutaneous nociceptors respond to potentially damaging stimuli and three main classes can be recognised. Mechanical nociceptors in the non-primate are innervated by C and Aδ fibres (Iggo, 1960; Burgess and Perl, 1967). Thermal nociceptors respond to high temperature (>42°C) and very low temperature (<10°C) and may also be excited by noxious mechanical input (Iggo, 1959). Bessou and Perl (1969) described a Polymodal nociceptor responding to chemical stimulants in addition to mechanical and thermal. Many algesic substances like KCl, bradykinin and serotonin are produced in pathological conditions of the skin and viscera. These substances sensitize or excite nociceptors and such effects have been studied on cutaneous afferents (Beck and Handwerker, 1974), visceral afferents (Lim, 1970) and skeletal muscle afferents (Mense, 1977). However the action does not seem to be specific since it has been reported that slowly adapting mechanoreceptors (SAII) can also be excited (Fjällbrant and Iggo, 1961). Prostaglandins seem to enhance the response of afferent fibres to bradykinin but do not have excitatory effects on their own (Chal and Iggo, 1977). It is also known that bradykinin

stimulates prostaglandin synthesis. There seems to be a positive feedback loop; prostaglandins increase the sensitivity of the receptors to bradykinin and bradykinin stimulates the synthesis of prostaglandins.

CUTNEOUS AFFERENT INNERVATION OF THE SPINAL CORD DORSAL HORN AND SOME MORPHOLOGICAL FEATURES

Rexed (1952 and 1954) divided the spinal gray matter into various laminae based on neuronal groupings as visualised in cyto-architectonic stains. Rexed's lamination is a useful guide but cytoarchitectonic studies do not give any information on dendritic arborizations and axonal connections which is important for understanding the complex structure and function of the neuropil.

Ranson (1913, 1914 a, b) gave the concept that the fine fibres are contained in the lateral division and the coarse fibres in the medial division of the dorsal root. Szentagothai (1964) confirmed this finding and recently it has been resolved even at the ultra-structural level, providing support for Ranson's original finding (Snyder, 1977). Morphological and physiological studies suggest that the fine fibres terminate superficially in the dorsal horn in laminae I and II (Ranson 1913, Christensen and Perl, 1970) whereas the thicker fibres terminate in lamina III and the dorsal horn ventral to it. There is some argument about the termination of thick fibres in the more superficial parts of the dorsal horn (Szentágothai, 1964; Scheibel and Scheibel, 1968, 1969; Kerr, 1975; Brown, 1977). Golgi studies (Scheibel and Scheibel, 1968)

demonstrated the presence of a plexus array similar to Cajal's flame-shaped arbors, formed by coarse cutaneous fibres in lamina II and III. With the advent of intracellular staining of physiologically identified afferents and neurones with the enzyme horseradish peroxidase (Snow et al. 1976; Jankowska et al. 1976), it has been shown that hair follicle afferents give terminals in laminae III and IV but not in lamina II (Brown, 1977) and also that the flame-shaped arbors are associated with these hair follicle afferents only. Brown (1977) made some important observations based on intracellular HRP staining on the rostrocaudal extent of hair follicle afferent arborizations, which follow closely the dendritic extent of spinocervical tract neurones in the sagittal plane. This rostrocaudal extent is larger in the lateral aspect of the spinal cord (1500 - 1700 mm for hair follicle afferents) than near the midline (500 - 700 mm for hair follicle afferents). Large cutaneous fibres that send their axons into deeper laminae III-VI, have wider terminal arborizations in deeper laminae which could contribute to the large receptive fields of the neurones in this region (Brown, 1981).

Recently (Light and Perl, 1979 a) using orthograde transport of horseradish peroxidase (HRP) gave evidence that Aδ fibres gave terminals in lamina I and the inner substantia gelatinosa (SGi) whereas the C-fibres gave terminals only in the outer and inner SG (SGo and SGi). In the same study superficial parts of the nucleus proprius have been shown to receive terminations from the large and intermediate fibres. By intracellular staining with HRP it has been shown that Aδ from mechanical nociceptors showed projections in lamina I, ventral parts of nucleus proprius (lamina V), ipsilaterally

and contralaterally in lamina I and the base of nucleus proprius (Light and Perl, 1979 b). In contrast to this projection of Aδ from mechanical nociceptors, a different projection pattern was seen for Aδ from D-hair as this showed terminations in the middle of lamina II (Light and Perl, 1979 b). It becomes apparent from these studies that terminations and projections are related to the sensory function and not to the diameter of the fibre. These striking features of functionally identified primary afferents and other morphological studies point to the fact that the marginal zone and SGo receive direct projections from cutaneous nociceptors whereas there is no direct projection from low threshold receptors to this region. Though there is no direct projection from non-nociceptors to lamina I yet this region can receive non-nociceptic input relayed via some SG interneurones as has been shown recently in the monkey (Price et al. 1979). It has been suggested from Golgi studies that the stalked cell in SGo can perform this function since the axon arborizes extensively in lamina I and the dendrites extend into SGo and SGi, which makes it a suitable candidate for receiving non-noxious input from small diameter primary afferent fibres and relaying it on to lamina I neurones. The suggestion that stalked cells perform the function of excitatory interneurones is a purely speculative idea based on Golgi studies, which could have been tested more thoroughly if the investigators (Price et al. 1979) had used intracellular HRP or some other staining technique in their study.

The cells in the marginal zone are large, horizontally arranged and the dendrites usually remain in a narrow area between Lissauer's tract and lamina I, occasionally entering lamina II (Réthelyi and

Szentágothai, 1973; Willis and Coggeshall, 1978; Light, Trevino and Perl, 1979).

Réthelyi and Szentágothai (1973) have described antenna-type neurones present in laminae III-V and the dendrites of these neurones have been shown to project into the substantia gelatinosa. The large antenna-type neurones located at the dorsal border of lamina IV are regarded as spinocervical tract neurones (Réthelyi and Szentágothai, 1973). However the dendrites of the physiologically identified spinocervical tract (SCT) neurones in laminae III and IV have been shown rarely to penetrate the substantia gelatinosa as revealed by the intracellular injection of HRP (Brown et al. 1977 b). This can either reflect the failure of the technique to reveal the finest dendritic branches which is not very likely or else that very few large antenna-type neurones constitute a part of the spinocervical tract. The SCT neurones as revealed by intracellular staining have a dendritic spread of up to 2 mm in the sagittal plane and also show dendrites projecting in the mediolateral and ventral direction (Brown, 1977; Brown et al. 1977 b; Fyffe, 1981). However dorsal column postsynaptic units in t his area (laminae III, IV, V) have a limited dendritic spread in the sagittal plane but possess dorsally directed dendrites ascending into lamina II, often entering lamina I as well (Fyffe, 1981; Brown and Fyffe, 1981).

The cells in lamina V are heterogenous, varying in shape and size as compared to lamina IV neurones. Scheibel and Scheibel (1968) have shown that the dendritic trees of lamina V neurones have little or no extensions in the longitudinal axis and radiate along dorso-

ventral and mediolateral planes. The longitudinal axonal plexus (Réthelyi and Szentágothai, 1973) of mainly primary afferent origin (Sterling and Kuypers, 1967) in laminae III, IV and superficial V changes its orientation to dorsoventral in lamina V which corresponds well with the dorsoventral orientation of the dendrites of neurones in this region (Scheibel and Scheibel, 1968). However some authors believe that the longitudinal axonal plexus extends through the entire nucleus proprius (IV-VI) (Kerr, 1975) and studies based on primary afferent degeneration (La Motte, 1977) reached a similar conclusion. The neurones in lamina VI are small as compared to lamina V and have their dendrites arranged in the mediolateral and dorsoventral direction with no extensions longitudinally (Scheibel and Scheibel, 1968).

SPINAL CORD NOCICEPTIVE NEURONES

Willis and Coggeshall (1978) have reviewed different classifications of dorsal horn neurones based on electrical and natural cutaneous stimulation used by several investigators. Iggo (1974, 1977) decided to simplify the classification of dorsal horn neurones into three broad categories based on electrical and natural cutaneous stimulation:

Class 1 — excited by non-noxious sensitive mechanoreceptors innervated by group II and III fibres (Aα, Aδ) but not by C fibres.

Class 2 excited by sensitive mechanoreceptive input and also by noxious input from thermal and mechanical nociceptors. A and C fibres supply these receptors. Many of these neurones which are very prominent in Rexed's laminae IV and V (Rexed, 1952, 1954) respond to input from sensitive mechanoreceptors only in the intact spinal cord but become additionally responsive to noxious stimuli when the spinal cord is blocked (Brown, 1971; Handwerker et al. 1975). This type of dorsal horn neurone is under strong segmental, tonic and phasic descending inhibitory interactions.

Class 3 neurones of this class are specific nociceptor driven and have been reported by various groups to be localised mainly in lamina I (Christensen and Perl, 1970; Cervero et al. 1976). Cervero et al. (1976) further subdivided this class into 3a and 3b. 3a are excited by Aδ fibres and respond to noxious mechanical stimuli whereas 3b in addition to Aδ receive input from C fibres and respond to noxious mechanical and thermal stimuli. Some neurones of this class are excited by group III and IV muscle afferents.

In a recent symposium (Brown, 1981) it was decided that there should be only one classification system rather than having several systems causing confusion and the conclusion was that Class 1 type neurones be called mechano-receptive. Class 2 or wide dynamic range and Class 3 will be known respectively as multireceptive and nocireceptive neurones. According to this new classification system, neurones examined in section two (II) were multireceptive and nocireceptive whereas only multireceptive neurones were investigated in sections three and four (III and IV).

There are two views on the transmission of nociceptive information and the first view lays emphasis on Class 3 or the specific nociceptor-driven neurones (nocireceptive) which are excited by the Aδ and C fibres and are present in lamina I (Christensen and Perl, 1970; Cervero et al. 1976). Some of these neurones project rostrally which has been shown by anatomical as well as electrophysiological techniques (Trevino et al. 1973; Willis et al. 1974; Kumazawa et al. 1975; Trevino and Carstens, 1975; Cervero et al. 1979). However Melzack and Wall (1965), Wall (1973) do not agree with this view, contesting that their exclusive response does not make these cells 'pain cells' since these neurones could represent 'the extreme of a broad distribution of cell thresholds'. Their disagreement seems to arise mainly with the psychological assumption of pain sensation associated with these cells rather than with the physiological specialisation of these cells (Melzack and Wall, 1965; Wall, 1973). There is considerable evidence that all nociceptors are innervated by the small diameter afferents (Iggo, 1959; Burgess and Perl, 1967) and these small diameter afferents remain in the superficial dorsal horn (Réthelyi and Szentâgothai, 1973; Light and Perl, 1979 a, b).

The second view is about the role played by Class 2 neurones in nociception since this type responds over a wide range of mechanical and thermal stimulus intensities (Mendell, 1966; Wall, 1960; Price and Wagman, 1970; Wall, 1973). These cells correspond to the transmission cells of the Gate control hypothesis (Melzack and Wall, 1965) which has already been discussed in a previous section. Class 2 neurones project into the spinocervical and spinothalamic tracts whereas the specific nociceptor driven or Class 3 project into the spinothalamic tract (STT) only*(Willis et al. 1974).

From the refractory period and electrical threshold analysis, Price and Mayer (1975) have demonstrated that these parameters of neurones lying in laminae IV-VI closely match with those of the ALQ-evoked pain in man. Based on these studies, it was concluded that pain may be signalled by the combined output of neurones in laminae I and IV-VI but that the activity of Class 2 neurones in laminae IV-VI should be sufficient for signalling pain. Some spinothalamic tract neurones in the monkey of wide dynamic range or Class 2 type (multireceptive) have been reported to respond to repeated noxious stimuli in a manner similar to the sensations evoked by the same stimuli in man (Price et al. 1978).

In the last few years it has been possible to record from the small neurones of the substantia gelatinosa and reports from various laboratories suggest that the SG is not a homogeneous physiological entity. Kumazawa and Perl (1976, 1977, 1978) recorded from the substantia gelatinosa neurones in the monkey and reported that the main input to these cells came from C fibres from polymodal noci-

* in the monkey

ceptors and mechanoreceptors. It has been reported that many of the SG neurones in the cat are excited by A and C fibres (Yaksh et al. 1977; Wall et al. 1979; Light et al. 1979) and their receptive fields vary from small to large (Wall et al. 1979). Some neurones in the SG show habituation (Yaksh et al. 1977; Hentall, 1977; Wall et al. 1979; Light et al. 1979) whereas others show prolonged discharge to sensory stimulation (Yaksh et al. 1977; Wall et al. 1979). Cervero et al. (1979 a) also reported a very small proportion of their unclassified neurones in the SG showing habituation. Some of the units reported by Wall et al. (1979) responded to hair movement and showed monosynaptic excitation from Aβ impinging on SG neurones. This finding is not consistent with the anatomical findings of Brown (1977) but is in accordance with the evidence provided by Proshansky and Egger (1977) who showed that Aβ fibres terminate in lamina II as well whereas the former has shown that these do not reach lamina II but end primarily in lamina III. Cervero et al. (1978, 1979 a, b) have given evidence about the presence of three different types of neurone in lamina II possessing persistent background discharge, inhibited by cutaneous stimulation, thus behaving like a mirror image of the three classes of neurones lying in laminae I, IV-VI (Iggo, 1974). A system of sensory interaction has been proposed in which SG neurones would exert a tonic inhibitory control on larger neurones in the dorsal horn which would be released during the stimulation period (Cervero and Iggo, 1978). There are differences on the extent of projection of SG neurones, Cervero et al. (1979 b) in their type of SG neurones reported a projection of two or three segments whereas Wall et al.(1979) in a physiologically different population from Cervero et al. (1979 a)

reported a rostral or caudal projection of less than 2.5 mm. Wall et al. (1979) have also reported inhibitory fields but always associated with excitatory fields unlike Cervero et al. (1979 a). Recently some evidence has been provided on the morphological features of physiologically identified SG neurones by using intracellular injection of HRP (Bennet et al. 1979; Light et al. 1979; Molony et al. 1980). It has been shown that all stalked cells and islet cells having dendritic arbors within SGo, either respond to noxious stimuli only or both to noxious and non-noxious stimulation and islet cells having the dendritic arborization in SGi respond to input from sensitive mechanoreceptors (Bennet et al. 1979; Abdelmoumene et al. 1981). Some investigators did not see any correlation between neuronal configuration and the functional features of their sample of SG but rather observed a close relationship between the location of the dendritic tree and the source of afferent excitation (Light et al. 1979). Those cells driven by nociceptors with myelinated fibres had dendritic projections in lamina I whereas cells responsive to nociceptors or thermoreceptors with unmyelinated fibres had dendritic arborization in SGo. The non-noxious mechano-sensitive neurones, receiving input from unmyelinated fibres, showed dendritic arbors in SGi (Light et al. 1979). The dendritic arborizations were found in SGi and superficial lamina III when the drive came from sensitive mechanoreceptors with Aδ fibres (Light et al. 1979). In this study, it has been reported that units driven from low threshold mechanoreceptors (Aδ and C) showed prolonged habituation whereas the other units excited by nociceptors and thermoreceptors did not. The thermosensitive units showed modulation by sensory stimulation and had marked background discharge.

SPINOTHALAMIC TRACT (STT)

The spinothalamic tract has been a classical pain pathway for over a century and the idea was based on the loss of pain sensation contralateral and caudal to a ventrolateral quadrant lesion (Spiller and Martin, 1912). Edinger in 1889 suggested a projection from the spinal cord to the thalamus but as a matter of fact, Gower in 1878, had given a similar suggestion relating temperature and pain sensation to the fibres present in the VLQ white matter. Some investigators using the Marchi technique failed to find or found very few spinothalamic tract fibres in the cat (Chang and Ruch, 1947; Morin et al. 1951) whereas other investigators using techniques such as Glees, Nauta or Fink-Heimer reported a moderate projection as terminal degeneration was localised in many diencephalic nuclei following cordotomy (Anderson and Berry, 1959; Mehler, 1969; Boivie, 1971; Jones and Burton, 1974). This tract is well developed in primates and man and is found in several mammalian species but there is a lack of information about this tract in non-mammalian vertebrate species (Willis and Coggeshall, 1978). There are several studies suggesting the presence of a lateral and ventral spinothalamic tract but it is possible that the central course of the cervicothalamic fibres might have been confused for STT projections from the contralateral dorsal horn, a view which was emphasised recently by Boivie and Perl (1975). Based on retrograde chromatolytic studies in man, two distinct pathways have been suggested; a lateral spinothalamic pathway taking origin from contralateral posteromarginal cells and a ventral pathway originating from the contralateral middle of dorsal horn (nucleus proprius) coursing more medially to the brain stem than

the lateral spinothalamic tract (Kuru, 1949). Mehler (1966) concluded from his comparative sub-primate studies that some fibres passing through the VLQ course medially in the brain stem and named it the paleospinothalamic pathway as this was thought to be phylogenetically older than the neospinothalamic tract. It was assumed that the paleospinothalamic tract is associated with pain and temperature (considering that these sensations are old) and terminates in the intralaminar thalamic complex whereas the neospinothalamic tract terminates in VPL. STT accompanies the spinoreticular and ventral spinocerebellar tracts in the spinal cord. Fibres ascending from the most caudal levels are most laterally placed and those from the rostral regions are medially placed (Hyndman and Van Epps, 1939; Weaver and Walker, 1941).

Cells of origin and physiological properties

Analysis of human and animal cordotomy material led several investigators to believe that a pain and temperature pathway present in the ventrolateral white matter of the cord and passing rostrally to terminate in the thalamus, takes origin from the large postero-marginal cells of the dorsal horn (Kuru, 1949; Morin et al. 1951) and from the centre of the dorsal horn nucleus proprius (Kuru, 1949). Morin et al. (1951) reached the same conclusion in their study on the cat and monkey since chromatolytic changes were seen in the ventral horn and its lateral border. Dilly et al. (1968) used the electrophysiological technique to study the cells of origin of the spinothalamic tract in the cat and rat cervical cord and found that the majority of the cells were concentrated in laminae V and VII.

Trevino et al. (1972) stimulated the medial lemniscus, at its entry into the thalamus for antidromic activation of STT neurones. Computer averaging was used to improve the signal to noise ratio. Most of their cells were located in lamina VIII and medial lamina VII. In this study, very few cells were found dorsal to lamina VI in IV, V and VI and none in lamina I. However in the lumbar cord of the monkey the neurones were found in t he contralateral laminae I, IV, V, VI, VII and VIII (Trevino et al. 1973). In their subsequent study, Carstens and Trevino (1978 a), using retrograde transport of HRP, showed similar results for the distribution of cells of origin of STT in the lumbar enlargement of the cat and monkey, contralateral to the injection site. However cells were also found to be labelled in lamina I in the lumbar enlargement of the cat. All the labelled cells were discovered in lamina I in L_7 in the cat when the injection site involved lateral half of the thalamus at the level of rostral VB-Complex including VL and Zona incerta. In the same study, ipsilateral projections were also demonstrated. Neurones receiving a powerful high threshold mechanoreceptive input from $A\delta$ and low threshold thermoreceptors have been shown to project to the contralateral VLQ (Kumazawa et al. 1975).

The spinothalamic tract, especially in primates, is responsive to light tactile and noxious input (Price and Wagman, 1971; Trevino et al. 1973; Willis et al. 1974; Albe-Fessard et al. 1974; Foreman et al. 1975). Willis et al. (1974) and Willis (1981) reported several different functional types of spinothalamic tract neurones as having response properties to cutaneous stimulation similar to Class 1, 2 and 3 neurones (Iggo, 1974, 1977). Visceral and muscle

input often converged on to Class 2 type or multireceptive spinothalamic tract neurones and some of the neurones also responded to proprioceptive or stimuli exciting deep receptors. Inhibitory receptive fields were rarely found and were located on the opposite limb and sometimes in the same position as the excitatory field on the contralateral limb. Similar inhibitory fields have been described by Poggio and Mountcastle (1960) for neurones in the posterior thalamus. STT cells projecting to the nucleus centralis lateralis (CL) showed wide receptive fields often bilateral and on intense stimulation included the fore and hind limbs, tail and face whereas the other group projecting to VPL or VPL + CL had small receptive fields on the contralateral hind limb (Willis, 1981).

Recently Price et al. (1978) reported four different types of spinothalamic neurone based on the range of their response to stimuli applied to receptive fields. Class 1 and 2 responded to innocuous stimuli, 3 responded to light tactile and deep pressure and pinch. Class 4 responded maximally to pinch with serrated forceps. Both the latter classes responded to noxious skin temperatures also. The most interesting finding in their study was that Class 3 and 4 neurones responded in a manner analogous to human pain sensations evoked by identical stimuli. First and second pain is evoked in humans by single electrical or heat pulse which also produced short and long latency responses in Class 3 and 4 neurones. Tactile stimulation produced prolonged discharge in Class 3 neurones and the same stimulus applied to the human foot produced after sensations.

C-fibre-evoked potentials and nociceptive activity have been recorded in the ventrolateral quadrant (Manfredi and Castelluci, 1969; Pomeranz, 1973). Though it has been easy to record single unit activity of the spinothalamic tract neurones in the monkey yet it has proved difficult to record from these units in the cat without using signal averaging. It seems that the spinoreticular tract is perhaps well developed in the cat as compared to the spinothalamic projection (Albe-Fessard et al. 1974). Dilly et al. (1968) recorded from the spinothalamic neurones in the cervical region which responded to noxious and non-noxious stimuli and ventrally located units were also discovered to respond to joint bending. It has been possible using signal averaging to record from these neurones in the lumbar region (Trevino et al. 1972; McCreery and Bloedel, 1975) and it has been reported that the units respond to input from sensitive mechanoreceptors only, high threshold mechanical nociceptors only, and both (McCreery and Bloedel, 1975). Some of the specific nociceptor driven cells in lamina I in the cat (Christensen and Perl, 1970; Cervero et al. 1978) and in the monkey (Kumazawa and Perl, 1975) have been shown to project contralaterally to the diencephalon in the cat (Dilly et al. 1968; Trevino and Carstens, 1975) and in the monkey (Willis et al. 1974; Trevino and Carstens, 1975; Albe-Fessard et al. 1974). It is apparent from these various investigations that recording from the spinothalamic tract neurones in the cat is still a problem and their physiological properties are beyond resolution. It is possible, perhaps, that the physiological role of the spinothalamic tract is to a large extent played by a spinoreticular projection which seems to be well developed in the cat. Recordings have been made from the spinoreticular neurones (Albe-Fessard et al. 1974). Responses of spinothalamic neurones

to tactile and nocuous cutaneous stimuli in the monkey give some indication of the functional significance of this pathway, which seems to have taken over from the spinoreticular tract unlike the cat.

STT terminations in thalamus

Boivie (1971) using a rigorous definition of VPL showed that the spinothalamic tract in the cat does not terminate in VPL unlike the monkey and this finding has been found true in subsequent HRP and electrophysiological studies (Trevino and Carstens, 1975; Holloway et al. 1978). The thalamic termination sites of STT are in the following nuclei:

Ventralis Lateralis	(VL)	:	in the cat (Boivie, 1971)
Posterior group	(PO)	:	in the cat (Boivie, 1971; Kerr, 1975) in primate (Kerr and Lipman, 1973)
Medial part of medial geniculate body	(MGm)	:	in the cat (Boivie, 1971; Kerr, 1975)
Zona Incerta	(ZI)	:	cat (Anderson and Berry, 1959; Boivie, 1971)
Centralis Lateralis	(CL)	:	in the cat (Anderson and Berry, 1959; Boivie, 1971)
Medialis Dorsalis	(MD)	:	in the cat (Anderson and Berry, 1959; Mehler, 1969)
Parafascicularis	(Pf)	:	cat (Anderson and Berry, 1959; Boivie, 1971)
Centrum Medianum	(CM)	:	(Bowsher, 1957; Nauta and Kuypers, 1958; Anderson and Berry, 1959)

Recently, using electrophysiological methods, Holloway et al. (1978) reported that STT fibres either terminate or axons pass through the following nuclear groups in the thalamus, the lemniscus medialis, n. centrum medianum, n. centralis lateralis (CL), n. ventralis posteromedialis (VPM), zona incerta (ZI), n. ventralis lateralis (VL), lamina medullaris externa and corpus geniculatum. Nuclei VL and CM contained the highest activity. Berkley and Mash (1978) reported that the nucleus centralis lateralis of the intralaminar complex in the cat contains the highest number of STT fibres and relatively few fibres were reported in VB and PO.

SEGMENTAL CONTROL OF DORSAL HORN NEURONES

Taub (1964) was the first investigator who reported segmental inhibitory effects being exerted on the spontaneous and evoked discharge of the spinocervical tract neurones in the cat on squeezing the toes of contralateral hind limb, contralateral and ipsilateral fore limb, pinching the nose, pinnae or tip of the tail and also tetanus of the forelimb radial nerves. Electrical stimulation of A fibres in the ipsilateral or contralateral plantar nerves inhibit the C-fibre evoked discharge of the spinocervical tract neurones (SCT) (Brown et al. 1974; Heath, 1978). The activity of Class 2 (multireceptive) and Class 3 (nocireceptive) neurones is suppressed on stimulation of sensitive cutaneous mechanoreceptors (Handwerker et al. 1975; Iggo, 1977). These findings lend support to the idea that the large fibre activity feeds an inhibitory mechanism into the dorsal horn (Melzack and Wall, 1965) and in fact stimulation of proximal ends of damaged nerves have been successfully used to relieve pain (Wall and Sweet, 1967; Meyer and Fields, 1972). Coarse cutaneous afferents are known to send axons into the dorsal columns and collaterals into the dorsal horn. The stimulation of dorsal columns inhibits the nociceptive responses of dorsal horn Class 2 (multireceptive) neurones in the cat (Hillman and Wall, 1969; Handwerker et al. 1975; Carstens and Trevino, 1978 b) and this stimulation also relieves pain in humans. Le Bars et al. (1979 a, b) studied the inhibitory effect of noxious stimuli applied on various parts of the body on the discharge of convergent (multireceptive) neurones in the rat. These nocuous stimuli inhibited the evoked response to both noxious and non-noxious skin stimuli as well as the discharge evoked on activation of $A\alpha$ and C fibres. These

authors call this phenomenon, 'Diffuse-noxious inhibitory control', (DNIC), since non-noxious stimulation did not produce inhibition. This phenomenon could be due to the involvement of supraspinal structures since it was not observed in the spinal preparations but on the other hand it may be more difficult to inhibit a cell having a high discharge rate on the interruption of tonic descending inhibitory control in the spinal preparation.

Opiate receptors (Pert et al. 1975; Atweh and Kuhar, 1977) and enkephalins (Hökfelt et al. 1980; Snyder, 1980) have been reported to be present in high concentration in the superficial dorsal horn. Iontophoretic and systemic administration of these peptides suggest that enkephalins and opiates produce selective inhibitory effects on the noxious input of multireceptive neurones in the dorsal horn (Duggan et al. 1977 a, b). However it is yet to be shown if enkephalins and opiates are involved in any of the segmental inhibitory mechanisms or tonic descending systems. DC stimulation in humans have been shown to be reversed by naloxone but that could involve supraspinal as well as segmental components thus providing no conclusive answer as to whether the endogenous opioids are involved in supraspinal or segmental mechanisms in these studies. The early and late inhibitions of dorsal horn neurones generated by volleys in mixed cutaneous afferents have been demonstrated to be mediated by glycine and GABA respectively (Game and Lodge, 1975).

DESCENDING CONTROL OF TRANSMISSION IN THE SPINAL CORD

Tonic descending influences were first realised when Sherrington and Sowton (1915) observed decreased stretch reflexes and enhanced flexor reflexes on transecting the spinal cord in a decerebrated animal. The possibility of the synaptic transmission to be under the regulatory and modulatory supraspinal influence was suggested by many workers (Head and Holmes, 1911; Brouwer, 1933; Peele, 1942). Fulton (1926) suggested that the internuncial neurones which form part of segmental polysynaptic reflexes might be under the inhibitory influence of descending pathways which might serve to regulate incoming afferent signals, suppressing some and deflecting others rostrally for integration at the higher centres of the nervous system. Inhibition of spinal monosynaptic reflexes from stimulation of the medial medullary reticular formation was shown by Magoun and Rhines (1946) and this inhibitory action was abolished by lesioning VLF.

Later on studies showed that the efferent gamma fibres of the ventral root influence the activity of muscle spindle afferents (Hunt and Kuffler, 1951) and that the central regions tonically activate the efferent gamma system (Granit and Kaada, 1952).

The higher centre control of dorsal horn interneurones was postulated by Austin (1952) and Lindblom and Ottoson (1955) on observing depression of cord dorsum potentials and polysynaptic reflexes on stimulation of the reticular formation. Hagbarth and Kerr (1954) studied the effect of repetitive stimulation of various brain

structures on the conducted afferent volley in the spinal cord and also showed tonic descending influences on spinal afferent pathways.

The tonic descending influences on interneurones relaying impulses in Ib, group II and III afferents were demonstrated by Eccles and Lundberg (1959). Holmqvist and Lundberg (1959, 1961) showed that the differential supraspinal control of cutaneous reflexes observed by Sherrington and Sowton (1915) depends on the integrity of the DLF alone. It has been suggested from further studies that a part of the tonic descending system is located in the serotonin containing midline raphe complex (Engberg et al. 1968 a, b). The dorsal reticulospinal pathway described by Engberg et al. (1968 c, d) was suggested to be playing, perhaps a lesser, part in exerting tonic descending effects. These descending influences on transmission in the spinal cord act presynaptically on primary afferents and postsynaptically on dorsal horn neurones (Lundberg, 1964; Carpenter et al. 1965).

The tonic descending inhibition of dorsal horn neurones has been studied by spinal cord cold blocking studies (Wall, 1967; Brown, 1971; Handwerker et al. 1975; Cervero et al. 1976) and a selective inhibitory control on the noxious input to Class 2 (multireceptive) neurones was revealed (Handwerker et al. 1975; Cervero et al. 1976). The tonic descending systems inhibiting polysynaptic inputs leaving monosynaptic input unaffected in SCT neurones have been suggested by Brown (1971). Selective inhibitory effects on noxious input observed by Handwerker et al. (1975) were explained, by assigning low synaptic security to polysynaptic input from C fibres as compared to the monosynaptic input from A fibres converging onto these neurones.

These tonic descending systems can inhibit spontaneous activity, noxious input in long ascending pathways (Brown, 1971) and can also switch the modality of some dorsal horn neurones (Wall, 1967).

Apart from the indication of the serotonin midline raphe complex being involved in the tonic descending systems, it is yet to be resolved which specific pathways and putative transmitters mediate these effects. The chemical nature of these pathways become further complicated in the light of the co-existence of transmitters (Hökfelt et al. 1980). The intrinsic and extrinsic factors and mechanisms responsible for the generation of activity in tonic descending pathways are yet to be explored.

There have been considerable efforts in finding areas in the various regions of the brain, in particular, the brain-stem, which play an important part in the descending control mechanisms. Stimulation produced analgesia (SPA) and morphine produced analgesia (MA) seem to be sharing a common neural mechanism, by activating descending pathways to the spinal cord and the spinal trigeminal nucleus caudalis. In the following pages, SPA, MA and enkephalins will be discussed followed by the anatomical evidence about some of the descending pathways and finally the descending control of dorsal horn neurones and the spinothalamic tract will be discussed.

STIMULATION PRODUCED ANALGESIA (SPA)

Pain inhibition by electrical stimulation of various brain structures has been shown in different species and, in particular, the medial brain stem structures produce profound behavioural

analgesia, which has been studied in the rat (Reynolds, 1969; Mayer et al. 1971; Mayer and Hayes, 1975), cat (Liebeskind et al. 1973; Melzack and Melinkoff, 1974; Mayer and Liebeskind, 1974; Oliveras et al. 1975, 1977, 1978), monkey (Goodman and Holocombe, 1975) and man (Richardson and Akil, 1973; Adams, 1976; Boethius et al. 1978; Guybels, 1979). The response to a wide variety of noxious stimuli seem to be inhibited by SPA. Oliveras et al. (1975) showed that stimulation of the inferior centralis nucleus in the cat produced powerful analgesia, modifying the response threshold for the jaw opening reflex and suppressing behavioural reactions elicited by noxious pinching applied to the tail and four limbs. Electrical stimulation of the medial brain stem has also been shown to inhibit the behavioural response to heating of the skin (Mayer, 1974; Soper, 1976), injection of chemical irritants and diverse clinical pain syndromes in man (Adams, 1976).

Stimulation produced analgesia does not result from a generalised sensorimotor or motivational deficit but is the result of a specific antinociceptive effect. The animal can respond to other sensory modalities during the period of analgesia (Mayer et al. 1974; Oliveras et al. 1974) and can perform other tasks such as eating (Mayer et al. 1974; Soper, 1976). SPA leads to a restricted peripheral field of analgesia (Mayer, 1974; Soper, 1976). It has been observed that some areas of the brain like the nucleus locus coeruleus which produce analgesia are also rewarding but these two phenomena are not interdependent. SPA depends on the integrity of the serotonin transmission whereas the intracranial self-stimulation (ICSS) requires catecholaminergic transmission as shown in the rat

(Sandberg and Segal, 1978). SPA from* PAG-PVG structures does not result from electrolytic lesions since electrolytic lesions produced at these sites did not diminish responses to noxious stimuli (Kelly and Glusman, 1968; Liebman et al. 1970).

MORPHINE-ANALGESIA (MA)

Microinjection of morphine into the PAG-PVG produces analgesia in the rat (Jacquet and Lajtha, 1973, 1974; Sharpe et al. 1974; Lewis and Gebhart, 1977) and in the monkey (Pert and Yaksh, 1974; Bennett and Hulsebus, 1976). It has also been reported that microinjection of morphine into the nucleus locus coeruleus produces analgesia in the rat (Yaksh et al. 1976). The analgesia produced by microinjection or systemic administration of morphine does not result from the local anaesthetic-like effect since microinjection of anaesthetics into the same regions do not produce analgesia (Yaksh et al. 1976). Hayes et al. (1978) observed the effect of morphine given systemically and transcutaneous electrical stimulation on the spinal cord nociceptive reflex and showed the presence of separate supraspinal pathways mediating MA (morphine analgesia) and shock analgesia. The DLF lesion abolished the former type of analgesia but did not affect the latter. There is a great degree of correspondence between sites producing MA (morphine produced analgesia sites) and stimulation produced analgesia (SPA) and opioid receptors and enkephalin distribution. However not all areas rich in the opiate receptors produce analgesia on microinjection, as for example, the rat striatum (Yaksh et al. 1976).

* Periacqueductal gray-Periventricular gray

Lesions blocking SPA and morphine analgesia (MA)

Lesions of the dorsal part of the lateral funiculus (DLF) have been shown to reduce or abolish SPA and MA produced on systemic administration of morphine (Basbaum et al. 1977). Morphine analgesia has also been shown to be blocked by raphe lesions, particularly the nucleus raphe magnus (NRM) (Saminan et al. 1970; Proudfit and Anderson, 1974; Yaksh et al. 1977). It has also been demonstrated that the analgesia produced on systemic administration of morphine was abolished by bilateral DLF lesions (Hayes et al. 1978).

Antagonism of SPA and MA by the opiate antagonist naloxone

Naloxone, the specific opiate antagonist, blocks SPA elicited by electrical stimulation of the nucleus centralis inferior (Oliveras et al. 1977). Microinjection of this opiate antagonist into the brain-stem blocks analgesia produced by the systemic administration of morphine (Tsou and Jang, 1964; Jacquet and Lajtha, 1974, 1976). Naloxone antagonises SPA from NRM in cats (Oliveras et al. 1974), from PAG in rats (Akil et al. 1976) and monkeys (Ruda et al. 1976) and from sites surrounding the third ventricle in man (Adams, 1976; Richardson and Akil, 1973). It has also been shown to reduce inhibitory effect of SPA on dorsal horn neurones responding to noxious stimuli (Bennett and Mayer, 1976).

Monoaminergic mechanisms involved in SPA and MA

The specific depletion of serotonin caused a reduction in SPA elicited from PAG stimulation while the elevation of serotonin levels

increased SPA (Akil and Liebeskind, 1975). Vogt (1974) showed that lowering the 5-HT concentration by 5, 6-dihydroxytryptamine blocked the analgesic action of morphine. Analgesia produced from the nucleus raphe magnus (NRM) disappears on continuous electrical stimulation and is restored by the administration of 5-HTP (Oliveras et al. 1978). LSD is also known to suppress the inhibitory effect of the dorsal raphe stimulation on certain dorsal horn interneurones (Guilbaud et al. 1973).

PHYSIOLOGICAL SUBSTRATE OF STIMULATION PRODUCED ANALGESIA (SPA) AND MORPHINE ANALGESIA (MA)

SPA - physiological substrate

Evidence is accumulating that brain stem stimulation-produced analgesia results from inhibition of nociceptive transmission at the level of the spinal cord dorsal horn and spinal trigeminal nucleus caudalis. The spinal cord nociceptive reflexes are inhibited by SPA (Mayer et al. 1971; Mayer and Liebeskind, 1974) and a similar effect has been seen on the jaw opening reflex (Oliveras et al. 1974). Though it cannot be argued that the inhibition of nociceptive reflexes means that the nociceptive transmission is blocked yet it gives an important indication that perhaps spinal withdrawal reflexes and the perception of pain share some common neural mechanisms in the dorsal horn. The anatomical evidence suggests that some dorsal horn neurones which send axons to VLQ also send collaterals to the ipsilateral ventral horn (Matsushita, 1969).

Behavioural analgesia is produced from the periaqueductal gray (PAG) stimulation, which also reduces the response of lamina V cells to noxious skin stimuli (Liebeskind et al. 1973; Oliveras et al. 1974). Spinal cord interneurones are also inhibited by dorsal raphe stimulation (Guilbaud et al. 1973). Electrical stimulation of the nucleus raphe magnus (NRM) inhibited the high threshold responses of laminae I, V, VI neurones, which was abolished by lesioning the dorsolateral funiculus (DLF) (Fields et al. 1977 b) and a similar effect has been demonstrated on STT neurones (Willis et al. 1977).

From the foregoing evidence it appears that there is an endogenous modulatory system in the medial brain stem, descending from PAG and NRM through DLF to dorsal horn neurones, which inhibits the nociceptive transmission and thus can produce analgesia.

PHYSIOLOGICAL SUBSTRATE OF MORPHINE ANALGESIA

(i) Supraspinal site of action.

(ii) Direct action on spinal cord.

(i) Supraspinal site of action

A similar mechanism of action as for SPA can be assigned to morphine analgesia whereby systemic administration or microinjection of morphine would activate a descending pain inhibitory system which would modulate the activity of dorsal horn neurones responding to noxious input.

However, so far only inhibitory effects of iontophoretic application of morphine and enkephalins have been reported in the various regions of the brain (Bradley et al. 1976; Gent and Wolstencroft, 1976; Fredrickson and Norris, 1976; Hill et al. 1976; Zieglgänsberger et al. 1976; Duggan et al. 1977; Randić and Miletić, 1978). Its excitant action has been reported on Renshaw cells (Davies and Dray, 1976; Belcher and Ryall, 1977). It is not necessary that an endogenous substance should have an excitatory action to activate a descending system. Morphine and enkephalins can do it if a tonically active inhibitory interneurone suppressing a descending pathway is assumed or else it could be that the technique might be biased to sample large neurones only. There is evidence to suggest that morphine and enkephalins do have excitatory actions. Systemic and intracerebroventricular administration of an analgesic dose of morphine and methionine enkephalin increased the spontaneous multiunit activity (MUA) in PAG (Urca et al. 1977). Anderson et al. (1977) have shown that systemically given morphine excites neurones in NRM including some which project to the spinal cord. Recently, evidence has been provided for the excitant effects of enkephalins and morphine in the hippocampus (Haas and Ryall, 1980; Nicoll et al. 1980b) spinal cord and olfactory bulb (Nicoll et al. 1980 b). It has also been shown that intravenous morphine did not produce a clear depressive effect on dorsal horn neurones in the decerebrate cat but in lightly anaesthetized rats it did

produce strong depression of some dorsal horn neuronal responses induced by the C-fibre activation (Besson, 1978).

(ii) Direct spinal action of morphine

Systemically administered morphine seems to exert some direct action on spinal cord dorsal horn neurones as it inhibits nociceptive responses of dorsal horn neurones in spinal animals, leaving tactile inputs intact (Kitahata et al. 1974; Le Bars et al. 1975). There have been several other reports of the naloxone reversible inhibition of nociceptive input to dorsal horn neurones in spinal cats by systemic administration of opiates (Besson et al. 1973; Takagi and Kawasaki, 1975; Le Bars et al. 1976). A similar action have been seen on axons in ascending tracts (Jurna and Grossman, 1976). Though there is evidence for the direct spinal action of morphine yet the descending pathways are implicated since microinjection into PAG produces reduction in nociceptive responses of dorsal horn neurones in the rat (Bennett and Mayer, 1976). Analgesic action of systemic morphine is also blocked by microinjection of naloxone into PAG and NRM.

THE NUCLEUS RAPHE MAGNUS (NRM), A COMMON MEDIATOR OF SPA AND MA

At present there is no evidence for a direct projection from PAG to the spinal cord but the periaqueductal gray (PAG) projects to

NRM (Rudda, 1976). Analgesic stimulation of PAG increases the multi-unit activity (MUA) in NRM (Olson and Liebeskind, 1975). Anderson et al. (1977) have shown that systemic morphine excites neurones in the NRM. Additionally it has been shown that NRM stimulation inhibited neurones receiving high threshold input in laminae I, IV and VI and this inhibition was markedly reduced by ipsilateral DLF lesion (Fields et al. 1977b). Willis et al. (1977) have shown a similar finding for STT in the primate. Evidence has been presented earlier that NRM lesions abolishes MA. Direct opiate injection into NRM does not produce analgesia (Takagi et al. 1976). This evidence suggests that systemic administration of morphine does not produce direct excitation of NRM and it is indirect via PAG-PVG as has been shown by Anderson and Fields (1977). From the evidence presented it seems reasonable to assume that NRM is the common mediator of SPA and MA.

NEUROHUMORAL SUBSTRATES

SPA - serotonin mediation

Common neural mechanisms underlie SPA and MA and monoaminergic transmission is thought to be playing an important part. LSD, a known serotonin antagonist, reduces SPA elicited from the dorsal raphe and inhibits nociceptive responses of lamina V cells (Newlon et al. 1976; Guilbaud et al. 1973). Analgesia from stimulation of the mesencephalic PAG is inhibited by p-CPA, a serotonin synthesis inhibitor and is increased by alteration of the serotonin level on 5-HTP administration (Akil and Mayer, 1972; Akil and Liebeskind, 1975). Tolerance develops on continuous electrical stimulation of the

inferior centralis which is also restored by 5-hydroxytrytophan (Oliveras et al. 1978). LSD did not reverse the antinociceptive effects produced by stimulation in regions other than the dorsal raphe which indicates that non-serotonin pathways may also be activated during SPA. Analgesia from NRM is produced in the cat (Fields and Basbaum, 1978) and rat (Olson and Liebeskind, 1975; Proudfit and Anderson, 1975) which is blocked by serotonin antagonists (Proudfit and Anderson, 1975).

Morphine analgesia - serotonin involvement

Depletion of serotonin with p-CPA, the synthesis inhibitor, partially blocks morphine analgesia (Tenen, 1968) and increasing the serotonin level potentiates morphine analgesia (Saarnivaara, 1969; Dewey et al. 1970; Sewell and Spencer, 1974). There is controversy surrounding the involvement of ascending and descending serotonin systems in morphine analgesia. The ascending serotonin system is implicated because the midbrain raphe lesions reduce analgesia produced by morphine and dorsal raphe stimulation potentiates it (Sanianin and Valzell, 1970, 1971). However there is strong evidence in favour of descending serotonin pathways as summarised below:

(1) Multiunit activity (MUA) increases in NRM on systemic opiate administration and lesioning this nucleus reduces morphine analgesia. (Proudfit andAnderson, 1975).

(2) DLF which contains descending serotonin fibres (Fuxe, 1965) on lesioning reduces analgesia produced by systemic administration of morphine (Price et al. 1976) or by microinjection into acqueductal structures (Murphin et al. 1976).

(3) Vogt (1974) has demonstrated that preferential destruction of the descending serotonin system blocks morphine analgesia.

Primary afferent depolarisation has been observed on systemic administration of morphine (Repkin et al. 1974) which was blocked by serotonin antagonists. Randić and Yu (1975) studied the effect of microiontophoretically applied 5-HT on dorsal horn neurones and observed depression of the nociceptive input in neurones in the superficial lamina of the dorsal horn. Microiontophoretic application of 5-HT and NA in the substantia gelatinosa, selectivity reduces the nociceptive responses of deeper dorsal horn neurones. Kenshalo et al. (1978) reported inhibitory and excitatory effects of 5-HT on STT neurones in the primate.

A common neural mechanism underlying SPA and MA is supported by the finding of the development of tolerance to SPA and cross tolerance between SPA and morphine analgesia produced by systemic morphine injection (Mayer and Hayes, 1975) or microinjection into PAG (Mayer and Murphin, 1976)

CATECHOLAMINE INVOLVEMENT IN SPA AND MA

Tetrabenazine (TBZ), a compound known to deplete all monoamines, almost completely abolishes SPA (Akil and Liebeskind, 1975). In the same study it was shown that depletion of norepinephrine potentiates SPA and dopamine receptor blockage reduces SPA, while stimulation with apomorphine potentiates it. Contrary to these findings it has been shown that electrical stimulation of the nucleus reticularis gigantocellularis produces analgesia and also inhibits the nociceptive responses of lamina V neurones. These inhibitory effects were abolished by TBZ and reversed by L-DOPA-a precursor of noradrenaline (NA) (Takagi et al. 1975). There is now enough evidence to suggest that in MA both serotonin and NA play an important part. Analgesia produced on microinjection of morphine into PAG in the rat is blocked by intrathecally administered α-blocker phentolamine (Yaksh, 1979) and similar evidence about the participation of α-receptors in analgesia has been given by Kuraishi et al. (1979 b). In their study analgesia was produced by intrathecally applied NA and blocked by pretreatment with phenoxybenzamine given intrathecally as well. The same group has shown previously that analgesic effects from microinjection of morphine into the reticularis gigantocellularis are blocked by phenoxybenzamine applied intrathecally (Kuraishi et al. 1979 a) and also that the normetanephrine levels go up in the spinal cord. Nociceptive responses of dorsal horn neurones have been reported to be blocked by iontophoretic application of NA (Belcher et al. 1978; Headley et al, 1978). The role of the catecholamine nucleus, locus coeruleus, in analgesia will be discussed in a separate section. These findings suggest that in addition to serotonin, spinal noradrenergic system plays a significant part in SPA and MA.

It seems reasonable to assume from this foregoing evidence that stimulation produces an endogenous opiate like substance, enkephalin, in the brain stem which activates the descending serotonin, noradrenergic and other pathways thus modulating nociceptive transmission in the spinal cord. An important part could be played by known and unknown peptidergic or other pathways as well (Hökfelt et al. 1979, 1980). This picture becomes further complicated with the evidence of co-existence of two transmitters in the same nerve terminal mainly a peptide and a classical neurotransmitter (Hökfelt et al. 1980).

DISTRIBUTION OF OPIATE RECEPTORS, ENKEPHALINS AND SUBSTANCE-P

Opiate receptors are present widely in the CNS (Pert et al. 1975) and the significance of the presence of opiate receptors in the CNS is even greater when seen in the light of an endogenous brain ligand for the receptor (Hughes, 1975; Kosterlitz and Hughes, 1975; Hughes et al. 1975). This new substance having properties similar to morphine was named enkephalin, (Leu-enkephalin and Met-enkephalin). Limbic structures contain the highest concentration of morphine receptors in the monkey and man; mid brain, particularly, the peri-acqueductal gray (PAG) contains moderately high levels of morphine receptors. The striatum is another area where morphine receptors are found in high concentration.

Enkephalins and opiate receptors overlap considerably in their distribution. Enkephalins are present in moderate levels in the mid brain, PAG, and low levels are reported for the cerebral cortex

(Simantov et al. 1976, 1977). Available evidence suggests that there are two peptidergic systems, one is a short chain peptide system, Leucine-enkephalin and Methionine-enkephalin which is widespread in the CNS and the other system is the long chain β-endorphin, a fragment of the pituitary hormone β-lipotrophin, centring around the hypothalamus-pituitary axis extending to the thalamus, mid-brain, and pons. Autoradiographic studies of Atweh and Kuhar (1977) and immunofluorescence studies of Hökfelt et al. (1977 a, 1980) provided useful information on opiate receptors and enkephalins. These studies show high concentration of opiate receptors (Pert et al. 1975; Atweh and Kuhar, 1977) and Met-enkephalin (Hökfelt et al. 1977 a) in the spinal cord laminae I, II, V and the spinal trigeminal nucleus caudalis. Lesion experiments suggest that enkephalin containing neurones in the dorsal horn are either interneurones or proprio-spinal neurones with nerve terminals in laminae I and II.

It has been shown that Met and Leu-enkephalin are contained in different neurones in the brain and intestine and two different receptor types have been suggested from binding studies, μ receptors possess an affinity for morphine and the delta receptor displays a preference for certain analogues of Leu-enkephalin (Snyder, 1980; Fields et al. 1980). It has been suggested that delta receptors regulate emotional behaviour and μ receptors mediate analgesic actions. The substantia gelatinosa has an equal distribution of both μ and δ receptors and also contains equal number of interneurones containing Leu and Met-enkephalins (Snyder, 1980). There seems to be a good overlap between opiate receptors, enkephalins and substance-P in the spinal cord, all being localised in the superficial dorsal

horn. There is evidence about the presence of opiate receptors on primary afferents and possibly on those afferents that contain substance-P and have their terminals in SG (Hökfelt et al. 1976; 1980; Snyder, 1980). The dorsal*horn contain both μ and δ receptors and partial destruction of small diameter afferent fibres after cutting the sciatic nerve causes a significant reduction in both μ and δ receptors (Fields et al. 1980). This is consistent with the previous findings on the depletion of opiate receptors after damaging primary afferents (La Motte et al. 1976; Jessel et al. 1979b). Capsaicin given to neonatal rats causes destruction of small diameter primary afferents (Jancsó et al. 1977), depletes substance-P from primary sensory neurones (Jessel et al. 1978; Nicoll et al. 1980 a; Nagy et al. 1980) and also reduces the opiate receptor density in the dorsal horn (Nagy et al. 1980). Substance-P has been reported to excite only those neurones that are excited by noxious stimuli (Henry et al. 1975; Piercey et al. 1980). There is substantial evidence concerning the transmitter role of substance-P which could be a transmitter for C-fibres and possibly Aδ fibres also that mediate nociception (Nicoll et al. 1980 a). Morphine analgesia could, in part, be produced by presynaptically inhibiting the release of the substance-P (Jessel and Iversen, 1977). Release of substance-P along with somatostatin has been shown in-vivo in mammalian primary afferents (Jessel et al. 1979 a). Distribution of substance-P, enekphalins, neurotensin and serotonin is very similar in the CNS. Cell bodies and nerve terminals of Met-enkephalin have been found in the PAG, NRM and the nucleus raphe pallidus (Hökfelt et al. 1977 a, 1980) and these areas are rich in serotonin (Dahlström and Fuxe, 1965) and substance-P (Hökfelt et al. 1980). The concept of a neurone producing

*root and dorsal

and releasing single transmitter, often referred to as Dale's principle (Eccles, 1957), is becoming doubtful in the light of the recent evidence about the co-existence of transmitters, a classical transmitter and a peptide contained in the same neurone. In the brain-stem in the rat, serotonin has been shown to co-exist with substance-P (Hökfelt et al. 1978, 1980; Chan-Palay et al. 1978), with the thyroid releasing hormone (TRH) (Hökfelt et al. 1980) and with TRH and substance-P (Hökfelt et al. 1980). Noradrenaline has also been reported to co-exist with somatostatin in the sympathetic ganglion and with enkephalin in the superior cervical ganglion (Hökfelt et al. 1980).

MECHANISMS OF ACTION OF OPIATES AND OPIOID PEPTIDES

There is considerable evidence about the inhibitory actions of opiates and opioid peptides in the brain stem in the cat and rat (Gent and Wolstencroft, 1976; Bradley et al. 1976; North, 1979) and in the cat spinal cord (Duggan et al. 1977; Belcher and Ryall, 1977; North, 1979; Zieglgänsberger, 1980). It has been suggested that both pre-synaptic (Jessel and Iversen, 1977; Konishi et al. 1979; Zieglgänsberger, 1980) and postsynaptic mechanisms are involved in producing the inhibitory action (Zieglgänsberger and Bayerl, 1976; Zieglgänsberger and Tulloch, 1979; Nicoll et al. 1980 b). Small doses of morphine and Met-enkephalin have been shown to cause hyper-polarisation of sural Aδ and C fibres but enhanced the depolarisation caused by primary afferents (Sastry, 1979). Enhanced primary afferent depolarisation (PAD) could in part contribute to the analgesic action of morphine.
It is interesting that morphine induces an increase in the levels of GABA (Kuriyama and Yoneda, 1978) which is known to cause an increase in the

the terminal excitability of primary afferents in the spinal cord (Curtis and Lodge, 1978).

The hippocampus is one site where consistent excitatory actions of enkephalins have been observed in-vitro (Haas and Ryall, 1980; Nicoll et al. 1980b) and in-vivo (Zieglgänsberger et al. 1979). Haas and Ryall (1980) in disagreement with Zieglgänsberger et al. (1979) and Nicoll et al. (1980b) have proposed a presynaptic facilitatory mechanism, similar to the one that Belcher and Ryall (1977) proposed for the facilitatory action of morphine on Renshaw cells whereas the others have proposed disinhibition as the mechanism of facilitation. In the isolated rat spinal cord (Suzue and Jessel, 1977) and in intact cats (Pomeranz and Gurvich, 1979), morphine and enkephalins produce inhibitory effect on DRPs. Recently, Nicoll et al. (1980 b) have shown inhibition of presynaptic inhibition by observing complete depression of DRPs by enkephalins in the isolated frog spinal cord. GABA is supposed to cause terminal depolarisation in primary afferents (Schmidt, 1971; Nicoll and Alger, 1979). The depolarisation action of GABA is not affected by enkephalins when DRPs are abolished, indicating that enkephalins are involved at some earlier step in the pathway (Nicoll et al. 1980 b). During the time of abolition of DRPs, hyperpolarisation is also observed and synaptic transmission from large myelinated primary afferents is either unaffected or enhanced (Nicoll et al. 1980 b). From this particular study it emerges that enkephalins depress GABA-ergic inhibitory pathways in the CNS but do not affect the action of GABA.

DISTRIBUTION OF MONOAMINES IN THE CNS AND DISTRIBUTION OF SEROTONIN TERMINALS IN THE SPINAL CORD

Dahlström and Fuxe (1964, 1965) studied the distribution of cell bodies of serotonin in the brain stem and nerve terminals in the spinal cord. The nucleus raphe pallidus, raphe obscurus and raphe magnus (NRM) and part of the reticular formation immediately surrounding the pyramidal tract at this level, contain serotonin cell bodies. The serotonin nerve terminals were reported to be driving through DLF for the dorsal horn and medial part of ventral funiculus for the ventral horn (Dahlström and Fuxe, 1965). Recently, topographical distribution of 5-HT terminals have been studied, using microdissection-microassay technique (Oliveras et al. 1977) and quantitative autoradiographic technique (Segu and Calas, 1978). The motoneurone area of the ventral horn contained the highest concentration of 5-HT and in the dorsal horn highest concentrations were found in the lateral part of the SG closely followed by the region surrounding ependymal canal. There was a remarkable decrease in the 5-HT level, seen after NRM lesions (Oliveras et al. 1977). Similar findings have been reported by Segu and Calas (1978) who demonstrated that the periependymal region, the sympathetic lateral-column, dorsal horn and motoneurone area of the ventral horn showed dense 5-HT innervation and contained the maximum number of boutons. However the dense innervation of DLF was not seen rather they were nearly absent.

ANATOMY OF MEDULLOSPINAL PATHWAYS

In this section a brief summary of the medullospinal pathways to the spinal cord will be given. The nucleus raphe magnus which has

already been shown to be the common mediator of SPA and MA, receives afferents from the spinal cord and cerebral cortex (Brodal et al. 1960 b) and gives efferents to the spinal cord and higher levels (Brodal et al. 1960 a). Similarly the raphe pallidus receives afferents and gives efferents. The adjoining reticular formation (nucleus reticularis gigantocellularis - Rgc) and the nucleus reticularis pontis receive afferents from the spinal cord and cortex (Rossi and Brodal, 1956, 1957) and give efferents to the spinal cord. The nucleus reticularis gigantocellularis has recently been shown to inhibit STT neurones in the primate (Haber et al. 1978).

Basbaum et al. (1978) have given evidence about three bulbospinal pathways from the rostral medulla using the autoradiographic technique. Following 3H Leucine injections into the rostral medulla, projections from the reticularis gigantocellularis (Rgc), the nucleus raphe magnus (NRM) and raphe magnocellularis (Rmc) have been shown as follows:-

(1) Rgc (nucleus reticularis gigantocellularis)

Terminal fields lie in the ipsilateral laminae VII and VIII via the ventrolateral funiculus (VLF), contralaterally in lamina VIII via the contralateral ventral funiculus.

(2) NRM

Bilateral projections coursing through DLF to the marginal zone, SG, lamina V, medial VI and VII.

(3) Rmc

Projections from this nucleus, which is lying lateral to NRM, overlap NRM projections but are ipsilateral via the DLF and VLF. In addition Rmc also projects to NRM and the raphe pallidus.

Evidence from Jouvet's laboratory, recently, showed a major projection from NRM coursing through DLF but they showed a moderate projection from the caudal part of NRM coursing through the VLF (Tohyama et al. 1979).

THE NUCLEUS LOCUS COERULEUS - INVOLVEMENT IN ANALGESIA AND ANATOMY OF A COERULEOSPINAL PATHWAY

The nucleus locus coeruleus (LC)

The interest in the nucleus locus coeruleus, which lies in the dorso-lateral tegmentum in pons, goes as far back as 1809 when Reil observed a streak of dark-bluish colouration imparted by the endogenous neuromelanin in the floor of the fourth ventricle in man. Russel (1955) established the locus coeruleus as a distinct nuclear entity based on Nissl-stain studies and it has been proposed that the development of LC parallels the elaboration of its cortical target areas (Tohyama et al. 1974, 1975). The frog rhombencephalon contains only twenty catecholamine neurones whereas the rat LC has 1600 (Swanson, 1976) and in the monkey it contains 7500 catecholamine

neurones. The presence of LC has been demonstrated in all the mammalian species studied so far including man (Amaral and Sinnamon, 1977).

LC has been implicated in such diverse functions as respiration, micturition, motivation, sleep, analgesia, reward and plasticity in the visual cortex. It seems the catecholamine containing LC and the serotonin containing mid-line raphe complex play contrapuntal roles. It has been suggested that the locus coeruleus (LC) imparts plasticity to the visual cortex whereas the serotonin raphe system switches off this neuronal plasticity which is best seen in the monocular deprivation experiments. The lag in development of cortical serotonin system behind the noradrenergic system from LC, could explain changes observed in monocular deprivation during the critical period and the gradual decline in this plasticity as the development progresses, (Pettigrew, 1978). Similarly the nucleus locus coeruleus and the serotonin raphe complex play an important role in sleep as bilateral lesions placed in LC suppress paradoxical sleep (PS) and have no effect on slow wave sleep (SWS). However the serotonin raphe system seems to maintain both PS and SWS and it has been suggested that serotonin mechanisms involved in slow wave sleep act as a 'priming mechanism' for triggering paradoxical sleep (PS) (Jouvet, 1968). Chu and Bloom (1973, 1974) recorded from LC in unrestrained cats and found that the majority of neurones displayed higher tonic rates of firing in paradoxical sleep than in slow wave sleep. Projections to the spinal cord have been demonstrated from both LC and the raphe complex and there can be complex interactions underlying the modulation of sensory and reflex transmission.

As compared to the tightly packed, compact arrangement of LC in the rodent and primate, the nucleus is diffuse in the cat (Amaral and Sinnamon, 1977). The dorsomedial portion of the nucleus is homologous to the LCd in the rat and extends for 3 mm in the cat, with the rostral one third being embedded in the periaqueductal gray (PAG) (Taber, 1961). The part of LC which is situated ventrolaterally to the dorsomedial part, is referred to as LCα by some authors (Maeda et al. 1973). The large fluorescent cells of caudal LCα merge ventrally and laterally in the sub coeruleus (SC) (Chu and Bloom, 1974). The catecholamine containing cell bodies in the cat have been reported to show a tendency to surround the brachium conjunctivum closely (Chu and Bloom, 1974).

It is perhaps not reasonable to divide LC in the cat into principal LC and the subcoeruleus (SC); the former contains tightly packed cells lying within the CG and the latter has neurones dispersed ventrolaterally through the dorsolateral tegmentum (Taber, 1961). This distinction is used and extended by several investigators in the cat (Maeda et al. 1973) and in the rat (Dahlström and Fuxe, 1965). It has been suggested that since there is no differentiation between LC and SC in Falck-Hillarp material as well as Nissl-stained sections, the sub-divisions should be based on the differential afferent and efferent connections (Jones and Moore, 1974; Chu and Bloom, 1974).

Presence of dopamine β-hydroxylase in the cell bodies in LC and the capacity to synthesize noradrenaline (NA) suggest that the cell

bodies contain NA and not dopamine (Amaral and Sinnamon, 1977). Neurotensin and substance-P containing cell bodies have also been reported to be present in LC. It is known that LC contains ten different terminals including 5-HT, Ach, NA, dopamine β-endorphin, Met-enkephalin and substance-P. There is a high density of opiate receptors in LC (Atweh and Kuhar, 1977; Pert et al. 1975, 1976). Morphine applied iontophoretically (Bird and Kuhar, 1977; Guyenet, 1980) or systemically (Korf, et al. 1974) in the rat has been reported to produce naloxone reversible depression of LC neurones. Guyenet (1980) has shown the depressant effect of morphine, NA, clonidine and GABA on identified coeruleospinal neurones in the rat. The locus coeruleus neurones respond to noxious stimuli (Guyenet and Aghajanian, 1977; Guyenet, 1980) and there is a dense innervation from substance-P fibres (Hökfelt et al. 1978). Intracerebral injection of substance-P causes turnover of brain NA (Magnusson et al. 1976). Guyenet and Aghajanian (1977) have reported the excitatory action of substance-P, Physalaemin, Eldoisin peptide and Acetylcholine (Ach) and the inhibitory effect of Met-enkephalin and L-norepinephrine on LC neurones. The inhibitory effect of NA seems to involve a collateral inhibitory mechanism, requiring the participation of α-autoreceptors (Aghajanian et al. 1977).

The locus coeruleus receives afferents from many places in the CNS and sends efferents back. There is anatomical evidence about the afferent-efferent connections of the locus coeruleus with the dorsal raphe, the nucleus raphe magnus, raphe pontis and centralis superior (Chu and Bloom, 1974; Sakai et al. 1977 a, b; Amaral and Sinnamon, 1977). NA cells in the LC that give rise to the dorsal pathway seem

to project to the dorsal raphe. The nucleus centralis superior receives projections from NA cells in the medial periphery of LC. The cells in the medial part of the sub-coeruleus innervate the nucleus raphe pontis and magnus in the cat (Chu and Bloom, 1974). Sakai et al. (1977 a) made a remarkable observation that the LC complex in the cat projected to the contralateral LC, innervating all the components but did not project to the ipsilateral reticular formation. Projections to PAG in the rat have also been reported (Amaral and Sinnamon, 1977). There is also a reciprocal projection between LC and the parabrachialis ventralis in the rat (Swanson and Hartman, 1975). Recently, evidence has been provided that the area of LC medial to LCα and medial part of LCα projects to the caudal and lateral two-thirds of the nucleus reticularis magnocellularis in the cat (Sakai et al. 1979). Projection from LC to the cerebellar cortex and the pontine raphe complex in the cat seems to be via axon collaterals of the ascending NA pathway (Chu and Bloom, 1974). In the rat, however, the ascending NE pathway gives off collaterals to the sensory relay nuclei in the thalamus and geniculate bodies (Amaral and Sinnamon, 1977). It appears that the axon of each neurone in LC breaks into an ascending and descending branch (Nygren and Olson, 1977) and reaches such targets as the caudal raphe complex and spinal cord with the descending branch. The ascending branch innervates areas such as the pontine raphe complex, sensory relay nuclei in the thalamus, sensory cortex, cerebellum, hippocampus, frontal cortex and visual cortex. Projections to such wide areas of the CNS as that would support the concept of a 'global regulatory role' for the locus coeruleus in the CNS.

Involvement of LC in analgesia

Electrical stimulation and microinjection of morphine into locus coeruleus produces profound behavioural analgesia in the rat (Sandberg and Segal, 1978; Yaksh and Rudy, 1978) and the analgesia produced by systemically given morphine is almost completely abolished by lesioning the LC (Sasa et al. 1977). LC lesions raise the nociceptive stimulus threshold whereas the dorsal raphe lesions seem to decrease it (Sasa et al. 1977). This finding is in disagreement with the findings of Hammond and Proudfit (1980) who showed that the lesions of LC and nucleus parabrachialis do not change the nociceptive threshold. SPA from LC was of the same magnitude as MA and was naloxone reversible. Higher current intensities were required for SPA as compared to the intracranial self-stimulation from LC (ICSS) (Sandberg and Segal, 1978). These investigators have reported that SPA depended on serotonin transmission whereas ICSS required the integrity of noradrenergic transmission and this latter finding that ICSS is produced from LC supports the earlier suggestion (Arbuthnott et al. 1970; Crow et al. (1972). SPA overlasted the period of stimulation for up to two minutes. It is hard to reconcile their finding (Sandberg and Segal, 1978) on the inability to block SPA with noradrenergic blockers but it is quite possible that SPA is indifferent to moderate interference with the catecholamines.

The part played by catecholamines in analgesia has already been stressed in a previous section but it shall be pointed out that there are conflicting reports as depletion of the whole brain noradrenaline levels have been shown to antagonise (Ayhan, 1972) or increase (Paalzow and Paalzow, 1975) morphine analgesia.

Chemical lesions induced by 6-hydroxydopamine of the dorsal noradrenergic bundle have been reported to potentiate morphine analgesia (Price and Fibiger, 1975). Recently, Hammond and Proudfit (1980) produced monosodium glutamate induced lesions, a method which is reported to spare the blood vessels, axons of passage and glia and to destroy only the cell perikaria. It was observed that lesioning LC and the nucleus parabrachialis ventralis (PBV) or lesioning PBV alone attenuated morphine produced analgesia. Based on these observations, the involvement of LC in MA has been ruled out. However this is not a very satisfactory conclusion since the investigators did not study the effect of lesioning LC alone. Some of these contradictory findings could result from species differences, tests for measuring analgesia, drug doses, pretreatment schedules, extent of lesions and the sensitivity of techniques for measuring transmitter levels.

It is quite possible that in MA both the catecholamine nuclei LC and PBV are involved. PBV in the cat has been demonstrated to have reciprocal connections with PAG (Sakai et al. 1977 a, b) and the dorsal raphe (Bobillier et al. 1976; Sakai et al. 1977 a). There is also a projection from PBL to the spinal cord (Tohyama et al. 1979). The neural mechanisms underlying the SPA and MA from LC have not been studied in detail at the spinal cord level.

Anatomy of a coeruleospinal pathway

A coeruleospinal projection from the locus coeruleus to all levels of the spinal cord has been demonstrated in the rat (Nygren and Olson, 1977; Guyenet, 1980), cat (Kuypers and Maisky, 1975;

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Hancock and Fougerousse, 1976; Tohyama et al. 1979), monkey (Hancock and Fougerousse, 1976; Kneisley et al. 1978) and in man (Papez, 1925). Though the catecholamine (CA) innervation of the spinal cord by descending fibres was first shown by Carlsson, Falck, Fuxe and Hillarp (1964) yet the supraspinal origin of these fibres innervating the dorsal and ventral horn has only been described in the last few years (Ross and Reiss, 1974; Nygren and Olson, 1977; Commissiong et al. 1978; Ader et al. 1979). Bilateral stereotaxic lesions placed in the LC decreased the CA nerve terminals up to 95% in laminae IV-IX and a partial disappearance of up to 25-50% in laminae I-III (Nygren and Olson, 1977). The remaining CA innervation of laminae I-III could be derived from the cell groups A_1-A_3 (Dahlström and Fuxe, 1965). It has been reported that the T-shaped branches of axons leave the locus coeruleus (LC) forming an ascending and descending bundle (Tohyama et al. 1974; Nygren and Olson, 1977) and suggestions have been made that all the cells in LC send an axon branch to the spinal cord. This is based on the evidence that the total number of CA fibres descending to the spinal cord are equal to the total number of cells in A_1-A_3 + CA cell bodies in the locus coeruleus and subcoeruleus in the rat (Nygren and Olson, 1977). LC innervates the entire length of the spinal cord through an ipsilateral ventrolateral coeruleospinal pathway. The concept of all cells in LC sending a descending axon to the spinal cord has been disputed recently in an electrophysiological study in the coeruleospinal projection in the rat (Guyenet, 1980). Crossed and uncrossed projections to the spinal cord from LC have also been demonstrated biochemically in the rat (Karoum et al. 1980). Dopamine β-hydroxylase activity decreases, ipsilateral to an unilateral lesion of LC (Ross and Reiss, 1974).

There seems to be much more evidence in favour of an ipsilateral projection than a bilateral projection. In a combined technique of retrograde transport of HRP with monoamine oxidase staining for NA, it has been reported that the ventral LC and SC give rise to a coeruleospinal pathway (Satoh et al. 1977). This finding has been confirmed electrophysiologically (Guyenet, 1980).

A coeruleospinal pathway in the cat has been described using retrograde transport of HRP by various laboratories (Kuypers and Maisky, 1975, 1976; Hancock and Fougerousse, 1976; Tohyama et al. 1979). Ipsilateral projections from LC and SC extending into an area ventrolateral to the brachium conjunctivum (BC), have been shown to descend to the lumbar cord. The neurones giving rise to this pathway seem to correlate very well with the CA containing neurones in this region (Jones and Moore, 1974). Chu and Bloom (1974) have reported that the CA neurones tend to surround the brachium conjunctivum, a tendency which is unique in the cat. An ipsilateral projection similar to the one reported by Kuypers and Maisky (1975) has been shown to descend to the lumbar spinal cord in the cat and monkey (Hancock and Fougerousse, 1976). A recent elaborate study on the spinal projections from the locus coeruleus in the cat at the cervical and thoracic levels has been reported from Jouvet's laboratory (Tohyama et al. 1979) and reached the following conclusions based on the retrograde transport of HRP:

1. Principal LC which is situated medial to the mesencephalic trigeminal root, has exclusively ipsilateral projection through the VLF and the caudal part of the nucleus seems to have a predominant projection.

2. The part of LC which is situated ventral to the principal LC and the medial dorsal half of BC, which the authors prefer to call LCα, showed a predominant projection through VLF, moderate projection through DLF and a small one through VF.

3. The locus sub coeruleus (LSC) showed a substantial projection, particularly from its caudal and medial part, through VLF. The lateral part of LSC projected through DLF and the ventral part through VF.

Recently, in an electrophysiological study on the rat Guyenet (1980) reported an unmyelinated pathway to the cervical spinal cord from the ventral division of LC and SC. The neurones showed slow but consistent firing and were inhibited by iontophoretically applied morphine, GABA, NA, clonidine and excited by Ach.

Sasa et al. (1974) have reported the inhibitory effect of LC on the spinal trigeminal neurones in the cat mediated by NA. A similar effect has been reported in the rat, with further evidence to show the existence of a 'feed-back loop' between LC and the spinal trigeminal neurones (Igarashi et al. 1978). Morphine has been reported to interfere with the LC induced inhibition of the spinal trigeminal neurones (Sasa et al. 1975).

Though there has been a lot of anatomical data supporting the presence of a coeruleospinal pathway in the cat yet it seems that there have been no efforts to elucidate the functional significance

of this particular projection. It is only recently, while the present investigation was in progress that facilation of monosynaptic reflexes (Strahlendorf et al. 1980) and the inhibition of spinal nociceptive afferents by stimulation of LC has been reported (Benelli et al. 1979). As yet there is no detailed analysis of the control of sensory transmission with particular reference to the nociceptive input by the locus coeruleus which would unravel the neural mechanisms underlying analgesia from this nucleus and throw some light on the functional significance of this pathway. It would be important to study the relative importance of the coeruleospinal and raphe spinal pathways. It has been shown that the midline raphe complex makes a partial contribution to the tonic control of reflex transmission but there are other studies, particularly by Duggan (1981), which show that serotonin is not involved. It is hard to clarify or reconcile these conflicting findings on the involvement of serotonin midline raphe complex in tonic descending inhibition. It seems reasonable to assume and implicate the coeruleospinal pathway in this inhibition since lesioning LC disrrupts the tonic components of paradoxical sleep (PS), like inhibition of neck muscle tone seen during PS. Noradrenaline inhibits the spontaneous and evoked discharge of spinal cord neurones (Engberg and Ryall, 1968; Belcher et al. 1978) and has a fairly selective inhibitory effect on nociceptive input of dorsal horn neurones (Belcher et al. 1978; Headley et al. 1978). The hind limb flexor reflexes have also been shown to depend on noradrenergic transmission (Lundberg, 1964). The general effects of LC stimulation are reported to be inhibitory on various CNS areas e.g. the hippocampus, cerebellum and cortex but excitation has also been observed (Olpe et al. 1980).

DESCENDING CONTROL OF DORSAL HORN NEURONES

Several areas of the brain like the cerebral cortex (Fetz, 1968; Brown et al. 1977 a), cerebellar nuclei and the reticular formation (Pompeiano, 1973) have been implicated in the descending control of sensory transmission. Along with many brain-stem areas, the sensori-motor cortex can also modulate nociceptive inputs, possibly through a relay in the bulbar raphe neurones that are known to project to the spinal cord (West and Wolstencroft, 1978).

A dorsal reticulospinal pathway descending in the DLF has been shown to inhibit interneurones and reflexes elicited by the flexor reflex afferents (group II and III peripheral afferents) (Engberg et al. 1968 b, c). DLF stimulation has been reported to inhibit transmission from high threshold receptors (Brown et al. 1973). A raphe spinal pathway coursing through DLF and exerting an inhibitory influence on the dorsal horn nociceptive neurones has been described (Basbaum et al. 1976; Fields et al. 1977 b; Willis et al. 1977). There is a marked reduction or abolition of this inhibition from nucleus raphe magnus (NRM) on lesioning the ipsilateral DLF (Fields et al. 1977 b; Willis et al. 1977). Electrical stimulation of the mid-brain PAG (oliveras et al. 1974; Carstens et al. 1979, 1980), the nucleus dorsal raphe (DR) (Guilbaud et al. 1973; Oliveras et al. 1974; Duggan et al. 1979) and lateral reticular formation (Zimmermann, 1979) inhibits the discharges evoked by noxious stimuli in the dorsal horn neurones. Fields et al. (1977 b) reported inhibitory effect from NRM on multireceptive (Class 2) and nocireceptive (Class 3) neurones. Excitation and mixed excitation-inhibition was also observed in some neurones.

Inhibitory effects evoked from dorsal raphe are blocked by LSD, a known serotonin antagonist (Guilbaud et al. 1973) but the site of action, spinal or supraspinal, is not known. LSD is also known to possess some 5-HT like agonist properties. Carstens et al. (1979 b) could not find any evidence for the involvement of enkephalins in the PAG generated inhibitory effects on the discharge evoked by nocuous stimuli. Morphine and enkephalins applied either iontophoretically in SG or given systemically selectively inhibit the nociceptive input of deeper neurones with little effect on non-noxious input (Duggan et al. 1977 a, b). Belcher and Ryall (1977) have reported the inhibitory effects of iontophoretically applied morphine on nociceptive cells and excitatory effects on non-nociceptive cells.

DESCENDING CONTROL OF THE SPINOTHALAMIC TRACT (STT)

It has been demonstrated that several ascending pathways are under the influence of the descending control systems (Holmqvist et al. 1960; Carpenter et al. 1965). In previous sections, it has been described that the nucleus raphe magnus (NRM) is the common mediator of SPA and morphine analgesia. The physiological substrate for these phenomenon is inhibition of dorsal horn neurones responding to noxious input. Some of these neurones project into ascending pathways. The spinothalamic tract plays an important part in nociception as well as thermal and tactile sensation (Kuru, 1949; Morin, 1951; White and Sweet, 1955). It might be that the descending control systems exert an inhibitory action on neurones receiving noxious input and projecting into STT and other pathways. This descending inhibitory control might be selective for the noxious input, leaving non-noxious tactile and thermal inputs intact.

The excitability of the primate spinothalamic tract neurones can be affected by pathways originating in the cerebral cortex. Sensorimotor cortical stimulation (hind limb area on postcentral gyrus) inhibits responses of STT neurones responding to low threshold mechanical stimulation, leaving high threshold mechanical input unaffected (Coulter et al. 1974). This preferential depression of non-noxious input to the spinothalamic tract might have bearing on the notion of "Corollary discharge" which is thought to modify the 'self-induced' sensory input anticipated to result from voluntary movement (Evarts, 1971). Some spinothalamic tract neurones are facilitated by corticofugal volleys, perhaps playing a part in monitoring the status of the spinal cord circuits involved in reflex activity (Lundberg, 1964).

Using signal averaging, McCreery and Bloedel (1975) showed reduction or complete suppression of STT neurones responding to high threshold mechanical stimulation, by electrical stimulation of the ipsilateral brain stem in the cat (Electrode pair in lower brain stem near the caudal end of the nucleus reticularis gigantocellularis). The reduction in responses could be seen from the contralateral brain stem stimulation also.

Stimulation in or near the raphe nuclei of the caudal brain stem inhibited STT neurones in the primate (Willis et al. 1977). Responses to both natural noxious and innocuous stimulation were inhibited but this descending inhibitory control was more effective for activity evoked by Aδ than by the large myelinated fibres. This pronounced inhibition from NRM was abolished on lesioning DLF,

showing that the descending myelinated pathway courses through DLF (Willis et al. 1977). The evidence for the medullospinal pathways coursing through DLF has been given by Basbaum et al. (1976, 1978).

Inhibitory and excitatory actions of stimulation of the ipsilateral and contralateral NGC on primate STT neurones were not abolished by DLF lesions (Haber et al. 1980). The DLF lesion sparing the descending inhibition is not surprising since Basbaum et al. (1978) have shown a bilateral projection to the spinal cord via VLF (ventral funiculus) from NGC. Centrally induced 'wind-up'* was seen in some cases, when excitation was observed on NGC stimulation (Haber et al. 1978). The responses to cutaneous stimuli noxious or non-noxious was either facilitated or inhibited on NGC stimulation (Haber et al. 1980).

5-HT is thought to be a neurohumoral substrate of SPA, MA and of the descending inhibition as has been demonstrated by several investigators. Jordan et al. (1978) observed the inhibitory effect of iontophoretically applied 5-HT on the high threshold and wide dynamic range spinothalamic tract neurones in the primate. It was proposed in their study that 5-HT acts post-synaptically since it reduced the excitant action of glutamate, which is thought to be acting on the postsynaptic membrane. The same investigators have also observed excitatory effects of 5-HT on STT neurones possessing deep receptive fields.

* Increased magnitude of responses evoked by successive stimuli of equal intensity.

MECHANISMS OF THE DESCENDING CONTROL OF TRANSMISSION IN THE SPINAL CORD - PRE AND POSTSYNAPTIC MECHANISMS

The primary afferent depolarisation (PAD) which is thought to reflect pre-synaptic inhibition, can be studied by recording the dorsal root potential (DRP), dorsal root reflex (DRR) or P-Wave which are manifestations of the PAD. It can also be investigated by direct recordings, measuring changes in the membrane potential (Schmidt, 1971) or by measuring the changes in the terminal excitability of primary afferents (Wall, 1958). The decreased threshold in the terminal region for antidromic excitation reflects PAD and consequent presynaptic inhibition whereas increased threshold (decreased excitability) reflects primary afferent hyperpolarisation as a consequence of which pre-synaptic facilitation would enhance synaptic transmission.

It has been suggested that GABA* is involved in presynaptic inhibition (Eccles et al. 1963). The reduction in DRP after topically applied GABA is assumed to result from its depolarising action on primary afferent terminals as well as its action on other sites of the PAD pathways (Schmidt, 1971). GABA applied electrophoretically has been reported to produce a decrease rather than an increase in terminal excitability (Curtis and Ryall, 1966). However the recent evidence from the same laboratory (Curtis et al. 1977; Curtis and Lodge, 1978) has demonstrated reversible depolarisation of group I terminals on electrophoretic administration of GABA. The depolarising action of GABA was shown to be reversibly reduced by a GABA antagonist, bicuculline. It has been pointed out that the failure

* γ-aminobutyric acid

to demonstrate depolarising action of GABA in the previous study could have resulted from the use of higher stimulating currents and concentrated solutions of drugs and amino acids. It has been shown recently that the depolarising action of GABA is generated by the activation of axo-axonic GABA receptors similar to the GABA-ergic axo-somatic synapses in the spinal cord (Curtis et al. 1980). In addition GABA has also been proposed to act on the non-synaptic receptors.

Presynaptic inhibition in the spinal cord has been produced from the cortex (Anderson et al. 1962) and many other areas (Schmidt, 1971). PAD has been produced on Ia, Ib and cutaneous afferents by stimulation of the dorsal midline region in the medulla and on Ib alone from the ventral medulla and cerebellum (Carpenter et al. 1966).

There is still paucity of information on the mechanisms of inhibitory effects produced from the brainstem areas on the nociceptive input of dorsal horn neurones. Hental and Fields (1979) reported increased intraspinal threshold (decreased excitability) for the C-fibres in the terminal region on electrical stimulation in NRM.

Carstens and Zimmermann (1981) have also observed decreased excitability of C fibres, caused by stimulation of PAG and the lateral reticular formation. Iontophoretically applied morphine and 5-HT also produced similar effects (Carstens and Zimmermann, 1981). These findings do not quite fit the current concepts of presynaptic inhibition (Schmidt, 1971) but can still cause inhibition of dorsal horn neurones by facilitating an inhibitory interneurone or by other

mechanisms (Hental and Field, 1979). However to resolve this question, would require recording intracellularly from the postsynaptic cell combined with measuring the excitability changes of the afferent terminal in the dorsal horn.

Engberg et al. (1968 d) have postulated that the dorsal reticulospinal pathway inhibits reflex transmission from primary afferents by postsynaptic inhibition of the first order neurones since IPSPs were recorded in some of the neurones receiving monosynaptic input. Jordan et al. (1978) have also suggested that 5-HT produces its depressant action on the postsynaptic membrane of spinothalamic tract neurones in the primate. However the evidence from this study is indirect since the investigators did not have intracellular recordings to show any IPSPs and based their conclusion on the evidence of inhibition of the glutamate induced discharge of STT neurones. These studies, so far, show the insecurity in their evidence for pre and postsynaptic mechanisms being involved in the descending control of transmission from the brain stem. It requires the combination of the Wall's excitability testing technique with intracellular recordings from the postsynaptic cell to give a satisfactory answer.

Other descending pathways

The anatomical evidence has been reported for the projection to the spinal cord of the ipsilateral medial reticular formation, contralateral ventrolateral part of the pontine reticular formation (Kuypers and Maisky, 1975; Hancock and Fougerousse, 1976), hypothalamus

(Kuypers and Maisky, 1975), ventral part of parabrachialis lateralis (PBL) and the Kolliker-Fuse nucleus (Hancock and Fougerousse, 1976; Tohyama et al. 1979) in the cat.

SUMMARY - SPA AND MA

Considerable evidence that has been presented on SPA and MA makes one fact outstandingly clear that in the brain stem exists an endogenous neural system, which on activation produces analgesia, possibly, by descending inhibitory control of spinal pain transmission neurones. PAG-PVG seem to be the one component of this central analgesic system since microinjection of morphine and enkephalins and electrical stimulation of these sites produce analgesia which is partially reversed by naloxone and serotonin manipulations. NRM, and the reticular formation (Rmc) seems to be forming a major outflow system to be spinal cord via DLF. Part of this analgesic system has been proposed to be mediated by the serotonin raphe spinal system (Mayer and Price, 1976).

PAG receives convergent somatotopically organised somatosensory input (Liebeskind and Mayer, 1971) and recently Price et al. (1978) have shown electrophysiologically that collaterals of STT neurones project to the medial brain stem central gray. It seems reasonable to assume that there exists a somatotopically organised negative feedback loop. Noxious input causing the release of an endogenous neuromodulatory substance, enkephalin, exciting neurones in the PAG which in turn activates the raphe spinal system. Both the ascending and descending serotonin systems play a role in this analgesic system.

Naloxone partially blocks SPA from PAG (Pert and Walker, 1976; Akil and Liebeskind, 1976) and it is more effective in blocking NRM stimulation produced analgesia (Oliveras et al. 1977). Endorphins are not present in significant amount in NRM. If SPA from PAG is mediated mainly via NRM then it should be completely reversed by naloxone. Fields and Basbaum (1978) have suggested the possibility of two independent pathways one via NRM mediated by enkephalins, reversed by naloxone and serotonin manipulations and the other via RMc which is not mediated by enkephalins. PAG stimulation could be acting on such structures in the reticular formation as RMc since the ventral central gray projects to the pontine and medullary reticular formation (Ruda, 1975). Alternative explanation would be that the medial brain stem stimulation could activate either enkephalin neurones or neurones postsynaptic to enkephalin neurone system whereas naloxone would reverse analgesia produced from enkephalin neurones only.

Apart from this previously described PAG-PVG and the raphe spinal system which is relatively well studied, the catecholamine story and the locus coeruleus require further studies to clarify the mysteries surrounding this area. Studies are required to investigate, the descending control from LC, the functional significance of the coeruleospinal pathway, physiological properties and significance of its afferent and efferent connections, neural mechanism underlying analgesia in relation to its efferent connections and the relative importance of the coeruleospinal projection as compared to the raphe spinal projection. The locus coeruleus could also mediate the analgesic effects of the dorsal raphe since DR projects only up to

the upper cervical segments (Tohyama et al. 1979) and there is evidence about DR projection to LC, at least in the rat (Segal, 1979).

AIM OF THE INVESTIGATION

The present investigation was carried out in the light of the literature reviewed and focused on the physiological properties of the spinothalamic tract neurones with particular reference to nociceptive transmission. The involvement of opiates in the segmental inhibitory control as well as the tonically active descending inhibition of nociceptive transmission was also investigated. The final part of the investigation throws light on the descending control of nociceptive transmission from the nucleus locus coeruleus.

SECTION II

ELECTROPHYSIOLOGICAL OBSERVATIONS ON SPINOTHALAMIC TRACT NEURONES

INTRODUCTION

The relevant literature on the spinothalamic tract has been reviewed in Section I. Various investigations employing anatomical and signal averaging techniques, have provided evidence about the existence of this tract in the cat. However, so far, recordings from individual spinothalamic tract neurones with microelectrodes in the cat have proved to be a daunting task as compared to the same projection in the primate which has yielded itself to analysis. This electrophysiological investigation was undertaken to record from individual spinothalamic tract neurones, to determine their projection to specific thalamic nuclei and the somatosensory properties of these cells which will be important for understanding the functional significance of this pathway in the cat with particular reference to nociception.

METHODS

Successful experiments were performed on 6 cats weighing 2.5 - 3.5 kg, anaesthetised with α-chloralose (60 mgm/Kg^{-1} i.v.) after induction with 4% halothane in a mixture of O_2/N_2O. The animals were paralysed with gallamine triethodide and artificially respired. Anaesthesia was controlled by allowing periodic recovery from paralysis, by observation of pupillary diameter and by monitoring arterial blood pressure to detect sudden surges to high values. Arterial blood pressure was continuously monitored and the results reported here are from preparations with a minimum mean pressure of 80 mm Hg. The end tidal CO_2 was continuously monitored and maintained between 3.5 - 4.5%. The body temperature was maintained

constant with an electric blanket thermostatically controlled by a rectal probe. Similarly, the temperature of the paraffin pool over the exposed surface of the cord was kept constant at 37°C. A lumbar laminectomy and a left craniotomy was performed. The dura over the cortex and spinal cord was split. The exposed cortical surface was covered with fine cotton wool soaked in normal warm saline.

Stimulation procedures

An array of 8 concentric bipolar stainless steel electrodes (200 µm tip diameter, 500 µm tip separation) was stereotaxically placed, according to the atlas of Jasper and Ajmone-Marsan (1954), in those thalamic nuclei known to receive axons of the spino-thalamic tract (Boivie, 1971; Holloway et al. 1978; Carstens and Trevino, 1978 a). The experimental arrangement was as shown in Fig. 2.1. The stereotaxic co-ordinates used in the majority of the experiments were: frontal, 6.5 - 9.5; L, 2 - 3; H, -2 - 0. The stimulating electrodes were arranged in four pairs built on a 1 mm grid. This arrangement effectively covered the centrum medianum (CM), ventralis posteromedialis (VPM), ventralis lateralis (VL). The rostral VB - caudal VL region which was demonstrated to receive projections from lamina I (Carstens and Trevino, 1978 a) received particular attention. Occasionally, the dorsoventral, mediolateral and rostrocaudal position of the electrodes was varied. In a few cases the lead of the thalamic stimulating electrode was fed into an amplifier through an isolation box in order to record evoked potentials from thalamic nuclei, generated by electrical and cutaneous stimulation of the contralateral hind limb. A constant

current of <200 μA - 300 μA, pulse width 300 μsec., was routinely employed for antidromic invasion of the spinothalamic tract neurones. For the two point determination of conduction velocity and estimation of STT projection compared to total projection through VLQ, another stimulating electrode (Ag-AgCl wire) was positioned on the 2nd or 3rd lumbar VLQ ipsilateral to thalamic stimulation. In a separate series of 13 experiments, the projections of specific nociceptive driven neurones in lamina I was investigated. Dorsal columns rostral to the recording site were lesioned. Bipolar silver ball electrodes were placed, rostral to the dorsal columns lesion, on ipsilateral dorsolateral funiculus (DLF) as well as the ventrolateral quadrant (VLQ). Stimulating electrodes were also placed on the dorsal columns, rostral and caudal to the lesion.

The hind limbs were shaved. The peripheral receptive fields were explored and mapped mechanically using a camel-hair brush, sharp and blunt probes and toothed forceps. A radiant heat source (Beck et al. 1974) was used for applying a controlled noxious and non-noxious heat stimulus.

Recording procedures

Single unit extracellular recordings were made in L6 and 7 contralateral to the thalamic and VLQ stimulation site, using 5M-NaCl or 4% Pontamine sky-blue in 0.5 M sodium acetate filled glass microelectrodes. Both the high and low impedance microelectrodes were used (5 to 100 mΩ). In some experiments, low impedance (5 to 10 mΩ) broken 5-M Nacl filled electrodes were used together with

on-line signal averaging. This improved the signal to noise ratio and facilitated location of areas showing antidromic activity generated by thalamic electrodes. This provided a useful index for searching the active region during single unit extracellular recordings. (Fig. 2.2). The microelectrode lead was fed into a high input resistance WPI amplifier, the output of which was further amplified and displayed in a conventional manner.

Criteria for antidromic invasion

The single units were classified as spinothalamic tract neurones if a fixed latency response to pairs and short trains of high frequency stimulation (200 - 500 HZ), was observed. The variability in the threshold latency for the antidromic units was less than 200 μsec. as has been described by other workers (Fuller and Schlage, 1976; Carstens and Trevino, 1978 b). In addition to the above tests, collision between the orthodromic and antidromic spikes was also employed. The collision between the orthodromic and antidromic spikes should occur during a specific interval which equals the antidromic latency plus one absolute refractory period (Trevino et al. 1972).

Data collection

The discharge (amplified action potentials) evoked by cutaneous stimuli was collected on a FM data tape recorder (Bell and Howell, CEC/Data tape). Antidromic impulses, their high frequency following, collision between the antidromic and orthodromic impulses were all

collected on the tape for further analysis.

Histological procedures

The animals were perfused at the end of the experiment with 10% formal saline through the arterial cannula. The brain was left in formal saline for 7-10 days for proper fixation and 40 μm serial frozen sections were cut and mounted on slides. Subsequently electrode tracks in these sections were examined and by interpolation stimulation sites were plotted on standard brain-sections.

The recording sites in the spinal cord were marked by electrophoretic deposition of the pontamine dye by passing a current of 5 μA for 5 minutes through the microelectrode. The resultant stain was localised in 40 μm frozen sections of the spinal cord which were subsequently stained with haemotoxylin-eosin.

RESULTS

Attempts to record from spinothalamic tract neurones succeeded in six cats. In these experiments 120 microelectrode penetrations yielded 252 units antidromically activated from VLQ but only 14 of these could also be antidromically activated from thalamic nuclei and satisfied the criteria, of fixed latency, high frequency following and collision for antidromic activation.

As outlined already, on-line signal-averaging was used to find the active regions for search. The paired search stimuli (50 - 300 μA) with an interstimulus interval of 2-3 m sec. were employed for

thalamic stimulation in order to generate antidromic activity which was picked up in an averaged signal (Fig. 2.2). This provided a useful guide in some experiments to search around an active region of antidromic activity from thalamic nuclei. Activity was described as antidromic if spikes showed constant latency (Fig. 2.2) and satisfied the other criteria.

In extracellular single unit recordings, collision between the antidromic spike from thalamic stimulation and an orthodromic spike elicited by cutaneous stimulation was demonstrated for five out of the fourteen spinothalamic tract neurones. Two of the units were lost before collision could be tested and in the remaining seven units collision was not tested. Collision has been demonstrated between the antidromic and orthodromic spike evoked by gentle stroking of receptive field as shown in Fig. 2.3 and occurred when the orthodromic action potential occurred just before the thalamic stimulus. The unit showed a constant latency to a pair of thalamic stimuli with a 2 m sec. interstimulus separation (Fig. 2.3). The unit was antidromically activated from the nucleus ventralis lateralis (VL) (Fig. 2.4 A) and was located in the medial part of lamina VII of the lumbar 7 segment of the spinal cord (Fig. 2.5). The threshold for antidromic activation was 35 μA.

Thalamic stimulation sites, threshold and current spread

Thalamic stimulation sites generating antidromic activity in the lumbar region were localised in the nucleus ventralis lateralis (VL) and centrum medianum (CM). The effective site of stimulation

was localised to a single thalamic nucleus in 50% of the units and five such effective sites were located in VL and two in CM (Figs. 2.4, 2.5).

All of the fourteen units excited antidromically from thalamic stimulation had stimulus thresholds varying between 10 and 150 μA, with a pulse width of 0.3 m sec., repeated at 1 HZ.

Current intensities employed (<200 μA) for antidromic activation showed that neuronal elements situated less than 1 mm from the tip of the stimulating electrode were excited. Movement of the electrode 1 mm dorsoventrally from the effective site did not produce an antidromic impulse at 200 μA (Figs. 2.4, 2.5). However occasionally antidromic activity could be generated from two sites 1 mm apart in the same nucleus but again dorsoventral movement of the electrode in tracking experiments revealed that it was not due to current spread as shown for VL and CM (Fig. 2.4). No effective sites were localised in the ventroposteromedialis (VPM) and the lateral half of VL.

Spinal cord location of spinothalamic tract neurones

Location of cells as shown by marking with pontamine sky-blue dye showed a ventral location of spinothalamic tract neurones mainly in lamina VII (Figs. 2.4, 2.5).

Projections of 39 specific nociceptive driven neurones localised in lamina I will be described separately.

Conduction velocity

The conduction velocity of spinothalamic tract axons was estimated by measuring the latency of the antidromic response to thalamic and ventrolateral quadrant stimulation and their respective conduction distances. As shown in a percentage frequency histogram (Fig. 2.6) majority of the axons (12 of 14) conducted at velocities >60 m/sec with an overall mean of 71 m/sec. These two point conduction velocity estimations are based on the assumption that the course of the axon is straight and not tortuous.

Multireceptive units with ipsilateral receptive fields

Spinothalamic tract neurones (8 of 14) were fully characterised whereas the remaining units were lost before any adequate mapping was done. Four of these eight (50%) spinothalamic tract neurones were responsive to both noxious and non-noxious stimulation of their receptive fields (Fig. 2.7 A). On adequate mapping, the receptive fields were located on the ipsilateral hind limb and displayed two distinct zones. The central zone of the receptive field was well defined and innocuous and nocuous skin stimuli applied with a sharp probe or pinching with serrated forceps elicited a higher rate of discharge as compared to innocuous stimulation. Surrounding the central zone was an ill-defined large area in which only noxious stimuli were effective and this zone sometimes extended over the entire limb.

Bilateral receptive fields of multireceptive neurones

Two of the 8 units had ipsilateral receptive fields as already described but in addition had a similar receptive field on the contralateral hind limb (Fig. 2.4 A, B). One of these units had an extensive receptive field covering both hind limbs, base of tail, ipsilateral abdomen, ipsilateral and contralateral forepaw and contralateral lower jaw as shown in Fig. 2.9 A. The inhibitory field was located on the ipsilateral thigh. This particular unit in addition to responding to brushing and pin-pricking also responded to flashing light into the ipsilateral and contralateral eyes. The unit also responded to moving vibrissae but did not respond to heat and auditory stimulation. Responses to pin-prick showed adaptation. The other unit did not have such an extensive receptive field (Fig. 2.4 B) but flexion of the ipsilateral and the contralateral foot produced inhibitory effect.

Spinothalamic tract cells with deep receptive fields

Two units had deep receptive fields. One of these units was excited by firm manipulation of deeply situated tissue located on the medial aspect of the ipsilateral thigh (Fig. 2.4 C). An adjacent area of the skin around the base of the tail was inhibitory. Both these units showed spontaneous activity and were not affected by hair movement or noxious heat. One of these units responded to low threshold mechanical stimulation applied on ipsilateral and contralateral thigh.

Specific-nociceptive units

Only one of the spinothalamic tract units showed characteristics of a specific nocireceptive neurone. The receptive field of the unit was small and located on the ventral surface of the ankle (Fig. 2.9 B).

The unit showed a mean frequency discharge of 13 HZ to a high threshold mechanical stimulation applied with serrated forceps and did not respond to innocuous stimulation as shown by the left hand horizontal bar (Fig. 2.7 B).

STT - noxious thermal and auditory stimulation

Only one of the fourteen units showed a response to noxious heat (45 - 53°C) applied to skin within the receptive field for high threshold mechanical stimulation (Fig. 2.8). It is hard to say what temperature was attained at the receptive field since only a qualitative heat source was used. In a few instances trial of auditory stimulation did not show any excitatory or inhibitory effect.

STT - inhibitory receptive fields and cutaneous stimulation producing inhibition

Inhibitory receptive fields were found for three of the spinothalamic tract neurones and were small compared to the excitatory receptive fields (Figs. 2.4 C, 2.9 A). Gentle stroking of inhibitory field on the ipsilateral base of the tail (Fig. 2.4 C) and thigh

proved to be an effective stimulus causing inhibition. In one unit no cutaneous inhibitory receptive field could be demonstrated but flexion of ipsilateral and contralateral foot produced inhibition. Two of these spinothalamic neurones possessing inhibitory receptive fields displayed extensive excitatory receptive fields on both hind limbs. No inhibitory fields could be demonstrated on the contralateral hind limb in a position corresponding to the ipsilateral excitatory fields.

Antidromic units from ipsilateral and contralateral VLQ - receptive fields and responses to cutaneous stimulation

A total of 252 units were antidromically driven from L_2 and 14 of these could be antidromically excited from thalamic nuclei as well and these spinothalamic tract units have already been described. The remaining 238 units antidromically invaded from VLQ had receptive fields on either the ipsilateral or on both hind limbs as shown (Fig. 2.10). Some units invaded antidromically from contralateral VLQ displayed extensive receptive fields covering both limbs (Fig. 2.10 A, B) and responded to noxious pinch and light mechanical pressure.

Inhibitory fields on the contralateral hind limb in an area corresponding to the ipsilateral excitatory receptive field were also demonstrated (Fig. 2.10 A). Stroking over the entire ipsilateral limb caused excitation whereas on the contralateral limb it produced short lasting inhibition. In this particular unit pinch applied between toe 2 and 3 produced excitation. The location of these cells was deep, similar to the spinothalamic tract neurones.

Some of the more superficially located units had smaller receptive fields and responded to brushing alone or brushing, pinch and heat (Fig. 2.10 C, D). The two illustrated units projected through the ipsilateral VLQ.

Projections of specific nociceptive driven neurones in lamina I

A total of 39 specific nociceptive driven neurones in lamina I were tested for projections but none could be antidromically activated from the thalamus. As outlined in the methods sections particular attention was paid to stimulate the rostral VB-caudal VL region which has been shown to receive projections from lamina I (Carstens and Trevino, 1978 a). Stimulation of VPM, VL and CM region also did not show any projections. Only three of these units were activated antidromically from spinal cord electrodes placed four or five segments rostral to the recording electrode.

In this sample, units responding only to mechanical nociceptive (18) and units responding, in addition, to thermal nociceptive input (21) were included in order to study if there was any trend for a particular type of noxious cutaneous input to project to thalamus. Only one of the specific nociceptor driven units was antidromically activated from contralateral VLQ, 28 mm rostral to the recording electrode. This unit followed high frequency and collision was demonstrated between the antidromic and orthodromic spike elicited by pinch applied to the receptive field. The unit was lost before it could be tested for antidromic activation from thalamus and its response to thermal nociceptive input. The remaining two units were

antidromically driven from ipsilateral VLQ and DLF as well as dorsal columns (DC). Since the unit was activated from electrodes both rostral and caudal to the DC lesion and required higher stimulus intensities its projection into DC was ruled out. Based on threshold of antidromic activation it was assumed that these two units projected into ipsilateral DLF since 4-10 times higher stimulus thresholds were employed for antidromic activation from ipsilateral VLQ.

All these specific nociceptor driven neurones received high threshold mechanical input and had small receptive fields as shown in Figure 2.11. Rarely some of these units were observed to respond to light tactile stimulation either after cold blocking or after repeated exposure to the noxious stimulus.

Excitation and inhibition from thalamic stimulation

As many as 300 units in this investigation showed excitation (96.7%) and inhibition (3.3%) on stimulation of thalamic nuclei for generating antidromic activity in the lumbar spinal cord. Post-synaptic activity was also recorded on stimulation of ipsilateral DLF, VLQ and contralateral VLQ. Thalamic and spinal stimulation generated one to several spikes and in a few units mixed excitation and inhibition was observed from thalamic stimulation. In some units in which postsynaptic activity was generated from thalamus and spinal cord (ipsilateral DLF, VLF and contralateral VLQ) it was observed that the first spike would follow up to 100 HZ stimulation without any shift in latency.

DISCUSSION

The majority of spinothalamic tract neurones in this investigation received input from mechanical nociceptors, sensitive mechanoreceptors and hair afferents. It is quite conceivable that these multireceptive neurones play an important part in the transmission of nociceptive information. Specific nociceptive driven neurones, displaying a discrete receptive field as demonstrated in this study can play a significant part in the onward transmission of signals containing information about the noxious stimulus and in the localisation of the stimulus. The combined output of multireceptive and specific nociceptive driven neurones or the output of multireceptive neurones alone have been implicated, in electrophysiological and behavioural studies, to signal pain (Price and Mayer, 1975; Price et al. 1978). It is perhaps not reasonable to assign a neurone the role which it plays in a particular sensation based only on electrophysiological studies in anaesthetised animals. The association of pain sensation with the specific nocireceptive neurones contains in itself the psychological assumption which is disputed (Melzack and Wall, 1965; Wall, 1973). The differentiation of the receptive field into two components based on the threshold intensity of stimulation which has also been observed in the primate (Price et al. 1978) can be a possible mechanism for the spatial radiation of pain sensation as proposed in the aforesaid study.

In this study spinothalamic tract neurones responding to heterosensory afferent input have been demonstrated. Somatic and visual stimuli converged onto the same multireceptive neurone whereas auditory

stimulation failed to elicit any responses. This observation is consistent with the findings of Wiesendanger (1967) who recorded responses of spinal cord neurones to visual stimulation. Previously reported ipsilaterally projecting spinothalamic tract neurones from the cervical region did not respond to visual or auditory stimulation. Various thalamic nuclei have been shown to respond to visual and auditory stimulation (Albe-Fessard and Besson, 1973) and nucleus ventralis lateralis (VL) which received projection from this particular neurone contains neurones that respond to somatic, auditory and visual stimulation. In a study by Massion et al. (1965) it has been reported that some neurones in VL respond to somatic stimuli only, some to somatic and visual or somatic and auditory only and still another population receives input from somatic, visual and auditory sense organs. The significance of this visual input may be to enhance the visually-directed movement repertoire of the animal and can be relayed through tectum or reticular formation which receives heterosensory input from several areas. The reticular formation is in a state of excitation in a chloralose anaesthetised animal.

Some of the multireceptive spinothalamic tract neurones displayed wide receptive fields consistent with the observations on ipsilaterally projecting spinothalamic tract neurones in the cat (Carstens and Trevino, 1978b) and a contralateral projection to CL in the primate (Willis et al. 1974; Willis, 1981). Similar responses and wide cutaneous receptive fields have been previously reported for neurones in VL (Massion et al. 1965), CL (Albe-Fessard and Kruger, 1962; Albe-Fessard and Besson, 1973), Posterior group (Poggio and

Mountcastle, 1960; Perl and Whitlock, 1961; Berkley, 1973) and in the reticular neurones (Wolstencroft, 1964). In fact some of the spinothalamic tract neurones projecting to VL were wide receptive field multireceptive neurones which could account for the somatosensory properties of VL neurones recorded by Massion et al. (1965). The somatosensory inputs of thalamic neurones cannot be accounted for on the basis of spinothalamic projection alone since various thalamic nuclei can receive varying afferent inputs from multiple places. Neurones in the reticular formation have been shown to possess wide cutaneous receptive fields (Wolstencroft, 1964) and bulbar and mesencephalic reticular formation have been shown to project to intralaminar thalamic nuclei (Bowsher, 1975; McBride and Sutin, 1976).

This investigation did not reveal any contralateral inhibitory receptive fields corresponding to excitatory receptive fields on the ipsilateral hind limb as has been reported in the monkey (Willis et al. 1974; Willis, 1981). It is perhaps possible that these neurones in the carnivore do not have contralateral inhibitory fields since the aforesaid neurones in the primate projected to VPL and this projection does not exist in cat. However this speculative possibility loses ground since this sample is small and the posterior group which receive spinothalamic projection (Carstens and Trevino, 1978 b) contain neurones possessing contralateral inhibitory fields corresponding to the ipsilateral excitatory fields (Poggio and Mountcastle, 1960). All the inhibitory receptive fields in this investigation were ipsilaterally located and sometimes situated adjacent to the excitatory field as in the primate (Willis et al. 1974).

The range of the conduction velocities (20 - 70 m/sec) of STT axons agrees with other reports in the cat (Dilly et al. 1968; Trevino et al. 1972) but is in disagreement with the findings in rat (Giesler et al. 1978) where slower conduction velocities (14 - 26 m/sec) were found. It is quite possible that the units antidromically driven from CM resulted from the stimulation of passing axons since the spinothalamic tract is known to terminate in VL but merely pass through CM.

Only 5.5% (14 of 252) of the antidromically activated units from VLQ could be identified as spinothalamic tract neurones. The remaining 94.5% (238 or 252) may have formed part of other ascending pathways such as the well developed spinoreticular tract (Fields et al. 1977a; Maunz et al. 1978a) but the possibility of these neurones forming components of the spinothalamic tract cannot be ruled out. Majority of the VLQ activated units were multireceptive, possessing bilaterally placed receptive fields. Some of these units had contralateral receptive fields as well. As has been already pointed out neurones displaying such characteristics as this have been recorded in the primate spinothalamic pathway (Willis et al. 1974) and are also found in the posterior thalamic nuclei (Poggio and Mountcastle, 1960).

Specific nociceptive driven neurones in lamina I had small receptive fields as reported already in the cat (Cervero et al. 1976) but only 8% of the total sample could be activated from 5 segments rostrally in the lumbar spinal cord. This finding is inconsistent with the findings of Cervero et al. (1979) who showed one third of

their neurones projected and Kumazawa et al. (1975) who reported that 23.8% of their sample projected to cervical levels in the cat and 42% in the primate.

The discrepancy between results presented here and those of Cervero et al. (1979) perhaps arose due to the fact that their study looked at projections only up to 3 segments rostrally whereas in this investigation the projections were looked at from 5 segments rostrally. Our differences from the findings of Kumazawa et al. (1975) arose because of strict criteria for antidromic activation in our study viz., constant latency, high frequency following (200 - 500 HZ) and collision. It has been relatively easier to study this particular projection in the monkey (Willis et al. 1974; Kumazawa et al. 1975; Handwerker et al. 1975 a). In the cat, although projection of lamina I to thalamus has been established anatomically, the present investigation failed to find any cells projecting to thalamus irrespective of the fact that the area receiving this projection (Carstens and Trevino, 1978 a) was given particular attention for stimulation.

As is evident from the evidence provided it has been tedious and extremely difficult to find spinothalamic tract neurones in the cat as has been frustratingly experienced by many other laboratories otherwise these neurones could have formed part of the investigations that follow this chapter. It is quite possible that this particular pathway in the carnivore is small as compared to the well studied projection in the primate. Carstens and Trevino, (1978 a) in their study using retrograde transport of HRP observed clusters of 2 to 5 cells in saggital sections separated by areas containing no labelled

neurones along the entire rostrocaudal extent of the segment. Branching of spinothalamic tract axons could also contribute since failure of antidromic invasion could occur at branch points. It is known that current from monopolar cathodal stimulation can block axon potentials if it is 8 times higher than threshold and closely situated small diameter axons can be stimulated whereas larger fibres may not be (Ranck, 1975). Considering this it seems that perhaps the location of the tip of the electrode is very important for activating a particular fibre type. Kumazawa et al. (1975) have suggested that antidromic activation may not occur in some fibres to a place which can be revealed in somadendritic recordings.

Thalamic stimulation apart from producing antidromic activation also caused an excitation (290 of 300) and inhibition (10 of 300) in neurones in this study. A faster conducting descending inhibitory pathway could hyperpolarise the cell thus preventing antidromic invasion. Postsynaptic activation whether generated via excitatory thalamo-reticulo spinal neurones (Mancia, et al. 1974; Wolstencroft, 1964) or the axon collaterals of the same neurone can possibly knock out an antidromic spike by collision. Some units postsynaptically excited from thalamus showed that the first spike would follow 100 HZ. This last argument though could have played a part yet does not sound convincing since depolarisation caused by descending pathways or collateral excitation should enhance the invasion of the cell by the antidromic spike.

It is not possible, based on our study, to say whether the small size of the STT in cat and its different distribution as compared to other species (Giesler et al. 1978; Trevino and Carstens,

1975) is an indication of its replacement by other ascending spinal pathways or whether despite its size it plays a similar physiological function. It is concluded with a speculative possibility based on the evidence presented that the spinothalamic tract in the cat can play a role in the transmission of noxious and tactile messages to the brain.

FIGURE 2.1

Experiment arrangement

The main experimental arrangement used in this investigation is shown. It shows some of the stimulation and recording procedures as indicated in the figure.

Abbreviations

CL : Centralis Lateralis

VL : Ventralis Lateralis

VM : Ventralis Medialis

VPL : Ventro Postero Lateralis

VPM : Ventro Postero Medialis

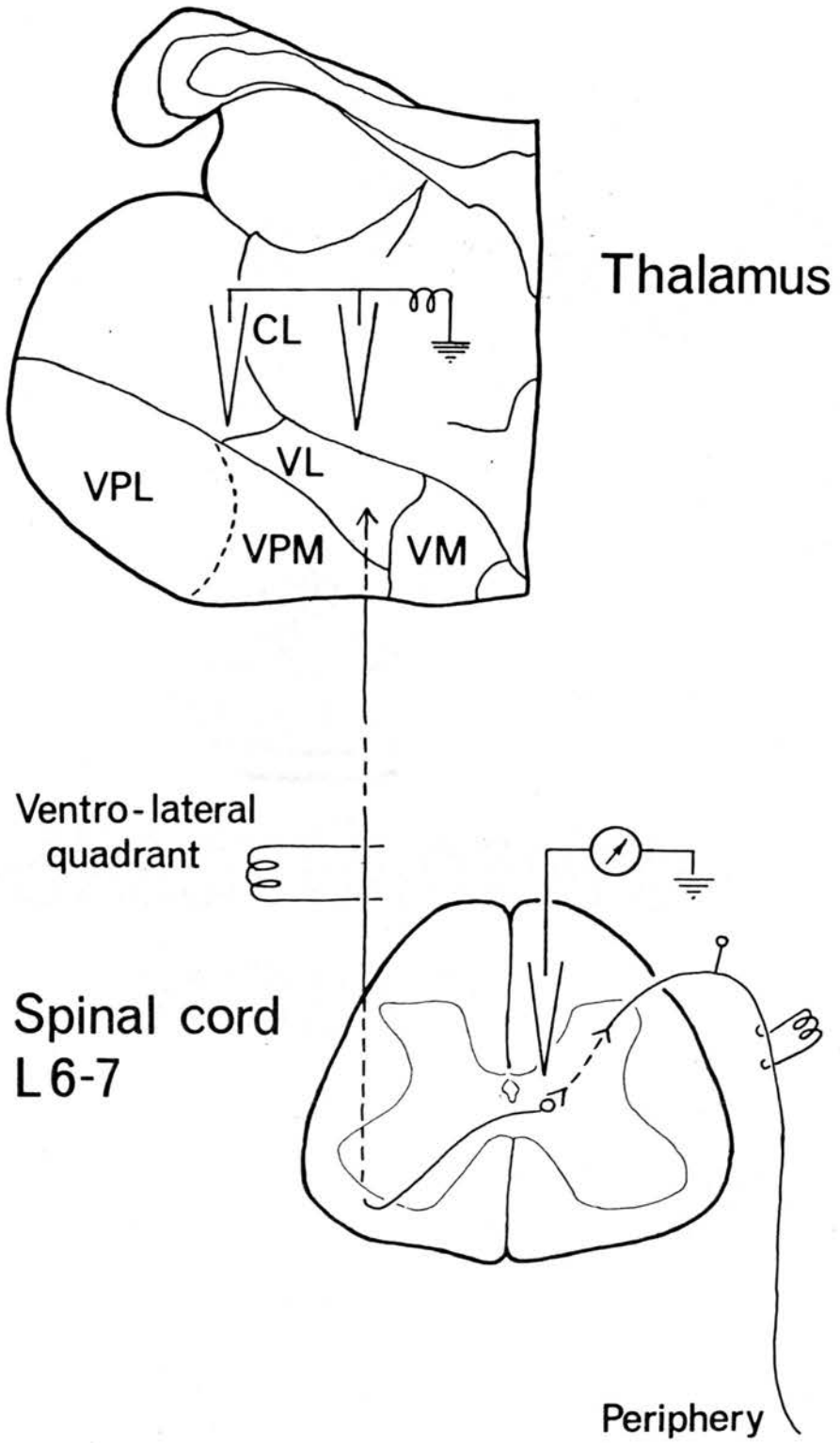

Thalamus
CL
VPL
VL
VPM
VM
Ventro-lateral
quadrant
Spinal cord
L6-7
Periphery

FIGURE 2.2

Andrimocic activity in an averaged signal

The figure shows averaged (150 repetitions) antidromic spikes generated from thalamic stimulation, recorded in L_7 segment of the spinal cord at different places. Paired stimuli, interstimulus interval 2 m sec., were used. The top figure shows antidromic spikes after the artifacts at suprathreshold. The bottom figure shows different antidromic spikes from above at threshold (50 μA) stimulation. These antidromic spikes, using signal averaging, were generated from the centrum medianum (CM). Later on it the same experiment, in extracellular single unit recordings, one neurone was found around the same area that projected to the centrum medianum (CM).

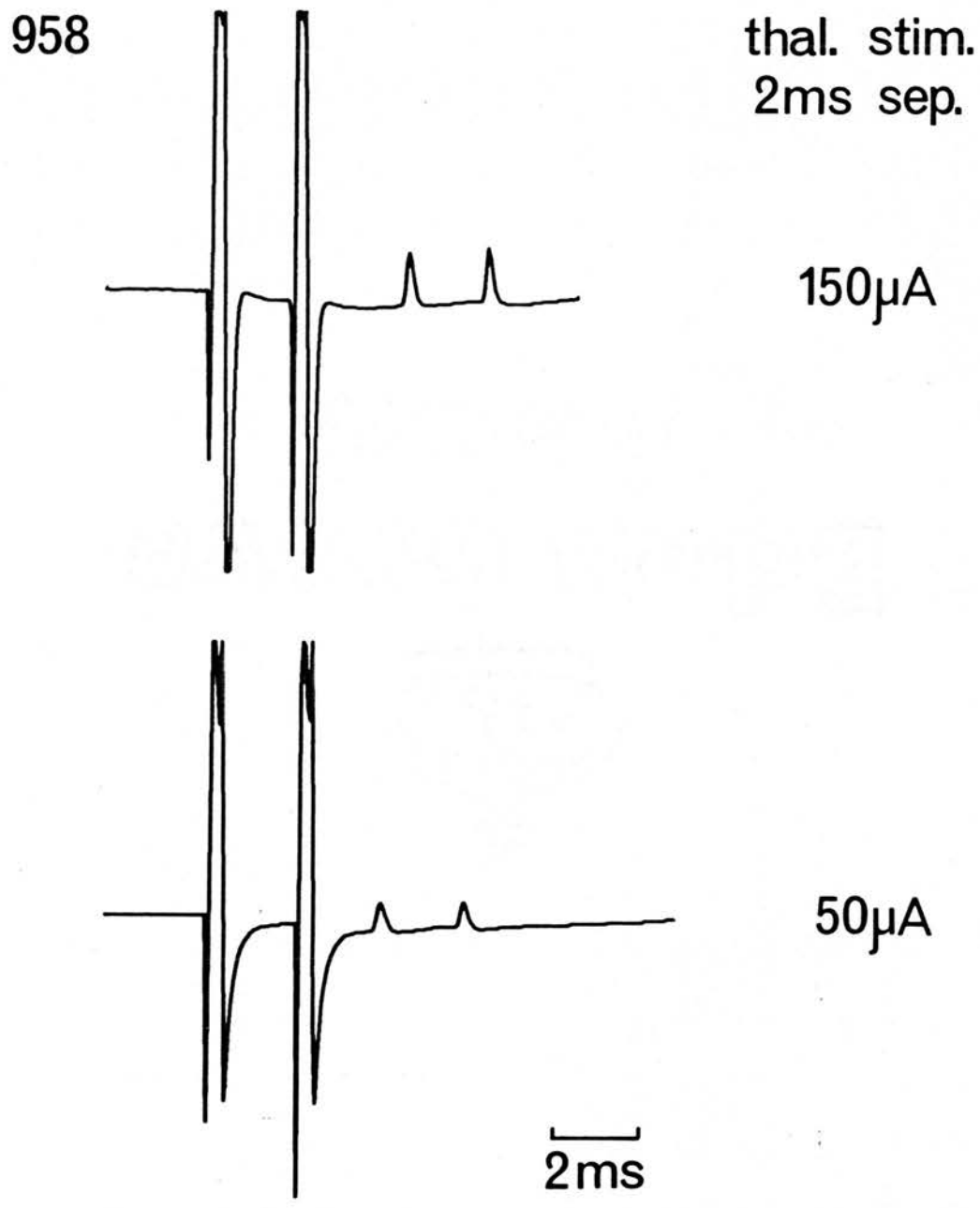

958
thal. stim.
2ms sep.
150μA
50μA
2ms

FIGURE 2.3

Antidromic invasion

The top figure shows the antidromic spike (r) having a constant latency, generated by paired stimuli (s) with an interstimulus interval of 4 m sec.

Collision: The bottom figure shows the collision between the antidromic and the orthodromic impulses. The top trace shows the antidromic spike and the middle trace shows cancellation of the antidromic spike (expected at *) caused by a precedent orthodromic impulse evoked by stimulation of the peripheral receptive field. S in the top and bottom figures is the stimulus artifact and r is the antidromic impulse.

Stimulus strength, 35 μA. The middle trace in the bottom figure is an overlap of 3 traces.

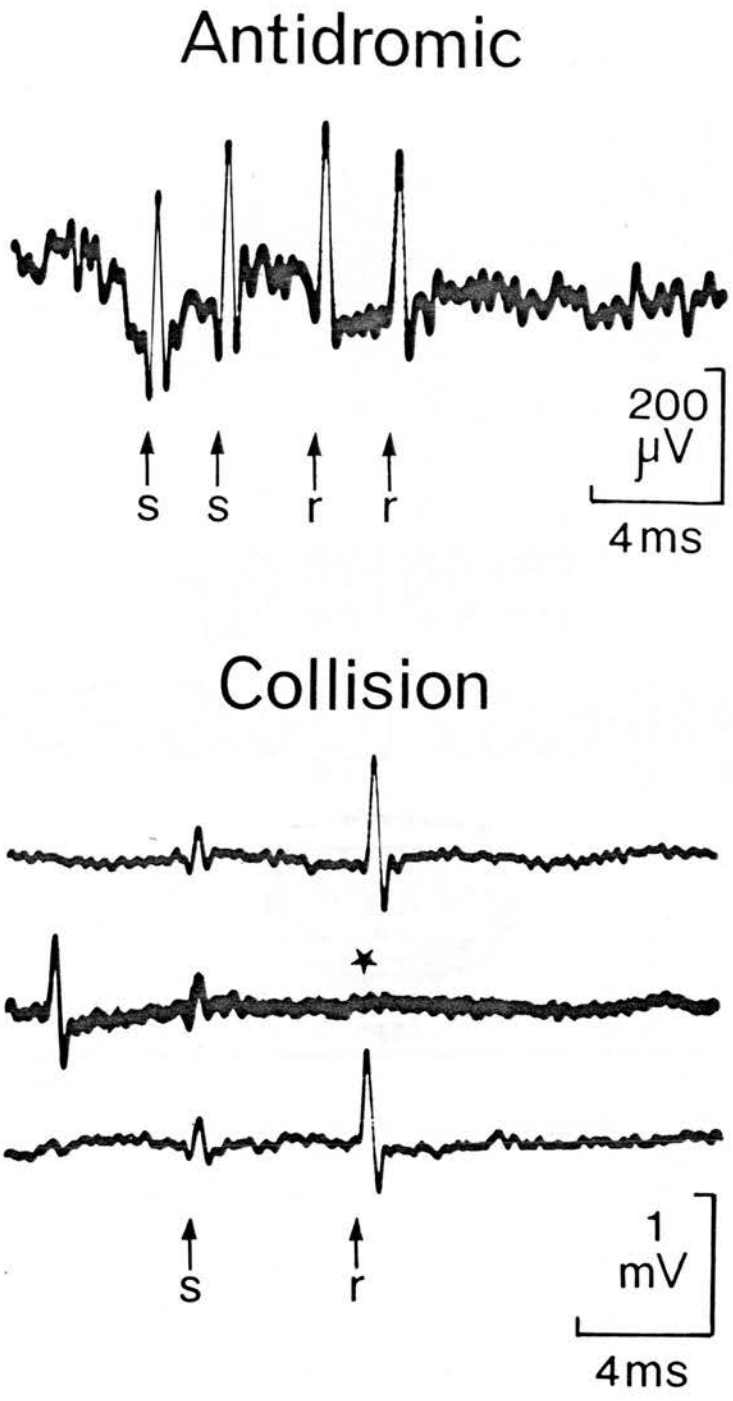

Antidromic
200
μV
s
s
r
r
4ms
Collision
1
mV
s
r
4ms

FIGURE 2.4

Thalamic stimulation sites, spinal cord recording sites and receptive fields

The figure shows the location of three different spinothalamic tract neurones (A, B, C middle figure), their corresponding thalamic stimulation sites (top) and receptive fields (bottom). All the three neurones were recorded in lamina VII in lumbar 7 segment of the spinal cord. Only one of these neurones (C) projected to the centrum medianum (CM) whereas the remaining two (A, B) projected to the ventralis lateralis (top figure A and B). The filled black circles represent sites which generated antidromic impulses on stimulation. The open circles represent areas which did not generate antidromic activity on using the same stimulation parameters. The threshold for antidromic activation from A, B and C were respectively, 35μA, 140 μA and <10 μA.

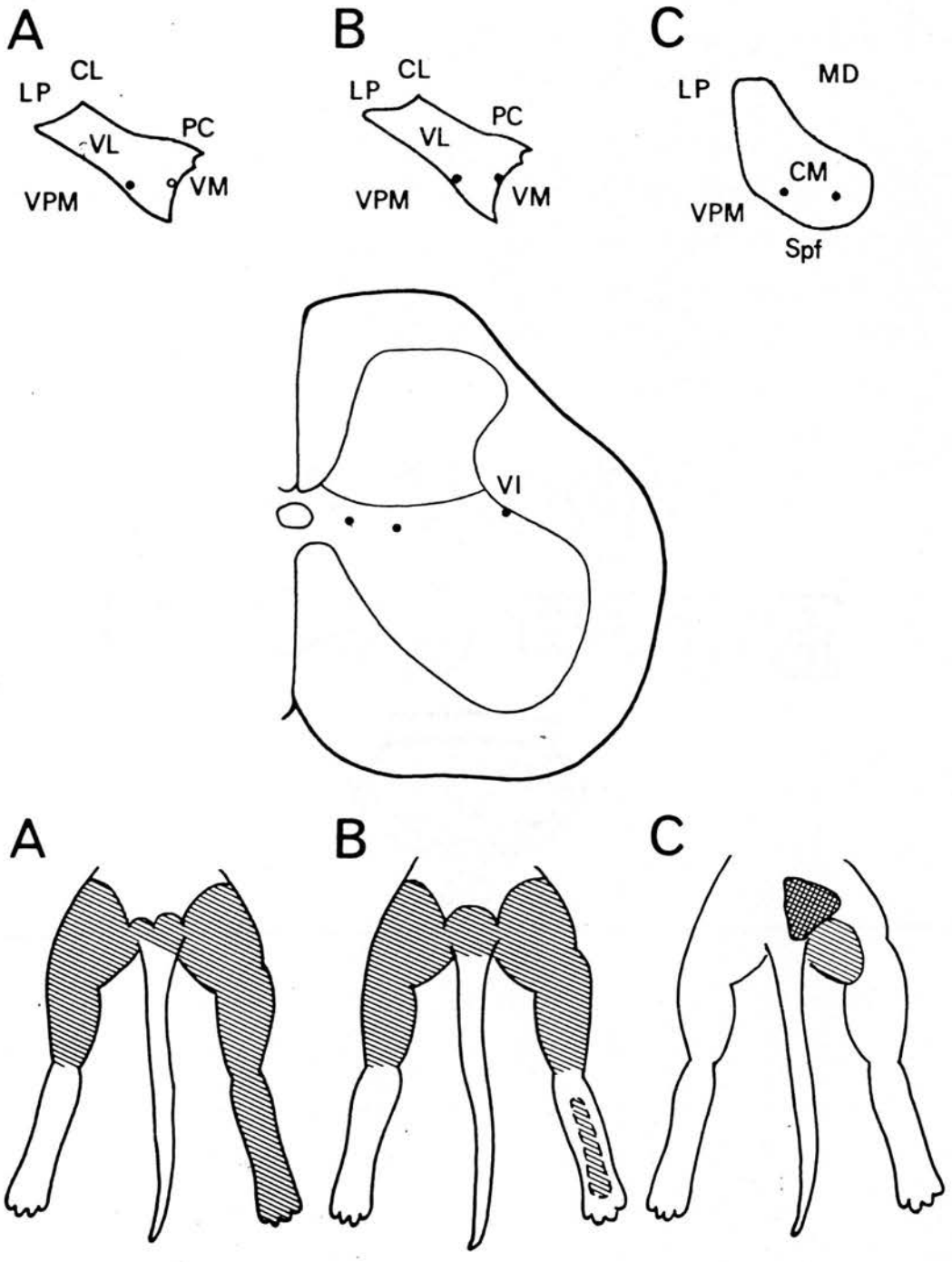
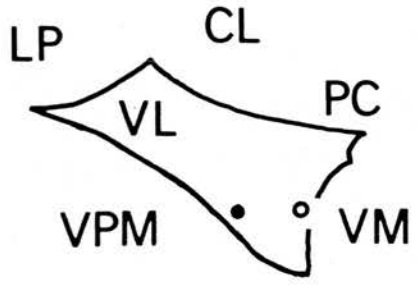
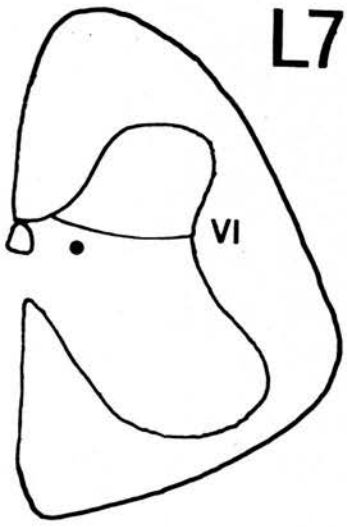

A
CL
LP
PC
VL
VM
VPM
B
CL
LP
PC
VL
VM
VPM
C
LP
MD
CM
VPM
Spf
VI
A
B
C

FIGURE 2.5

This figure shows the non-noxious excitatory receptive field, the spinal cord location and the thalamic site evoking antidromic activation. Brushing the receptive field (hatched area) was an effective stimulus. This unit had an excitatory receptive field on the ipsilateral thigh and back of tail where noxious pinch or pin-prick was the effective stimulus. At top left the numbers represent the stereotaxic coordinates and the threshold (35 μA) for antidromic activation.



A 9·5 ; L 3·0 ; H 0 ; 35μA



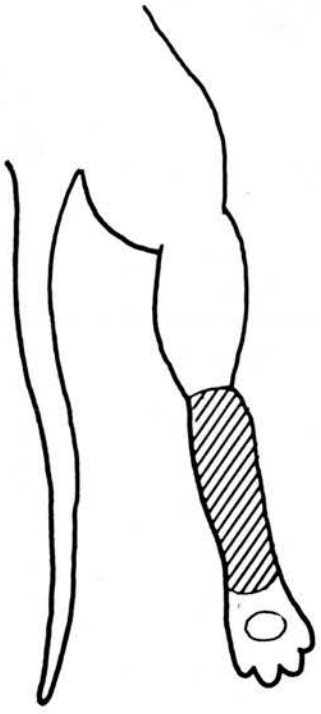


FIGURE 2.6

Conduction velocity

A percentage frequency histogram of the estimated conduction velocities of spinothalamic tract neurones. The sample size is, n = 14. The majority of the axons conducted at conduction velocities >60 m/sec with an overall mean of 71 m/sec.

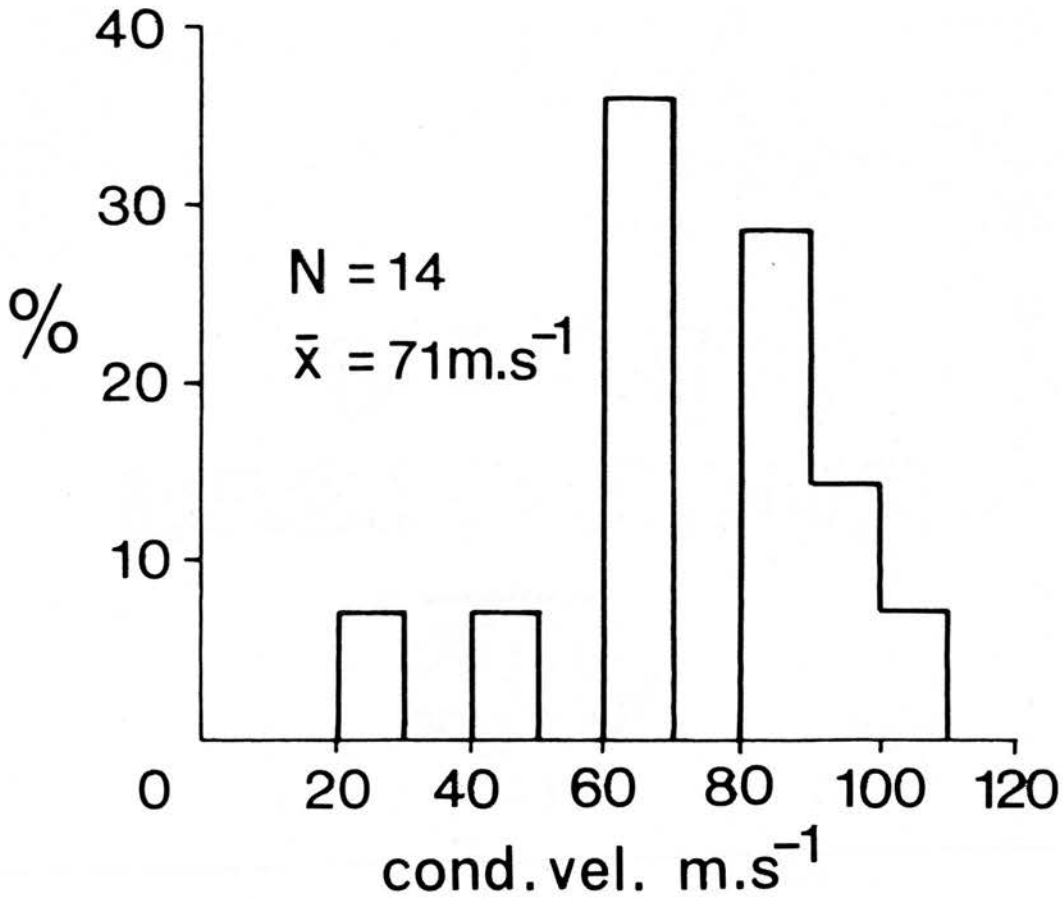

40
30
20
10
%
N = 14
$\bar{x}$ = 71m.s^{-1}
0
20
40
60
80
100
120
cond. vel. m.s^{-1}

FIGURE 2.7

Response of spinothalamic tract neurones

A : Shows the evoked discharge of a spinothalamic tract neurone elicited by gentle brushing and stroking (left-hand horizontal marker) and firm pinch (right-hand horizontal marker) applied to the receptive field.

B : Unit responding to noxious mechanical stimulation only (right-hand horizontal marker). The non-noxious stimulation did not evoke any discharge (left-hand horizontal marker).

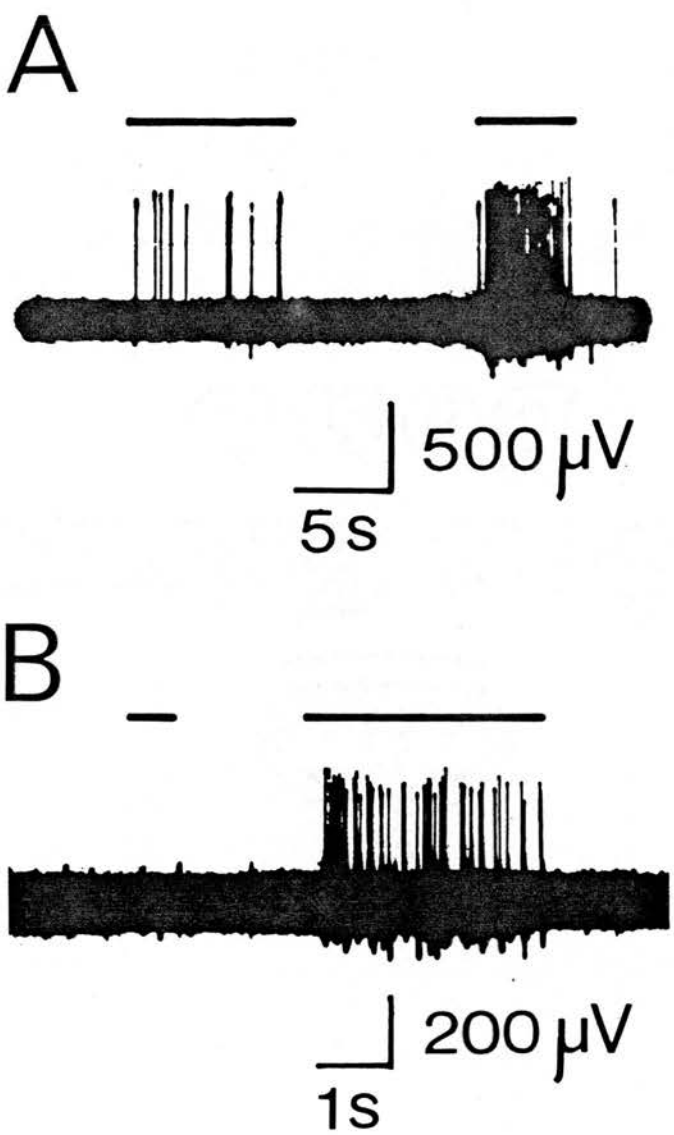

A
500 µV
5 s
B
200 µV
1 s

FIGURE 2.8

Response to heat

Frequency histogram of the response of a spinothalamic tract neurone evoked by the application of heat on the cutaneous receptive field. The neurone had background ground discharge and the application of heat stimulus is indicated by the horizontal marker.

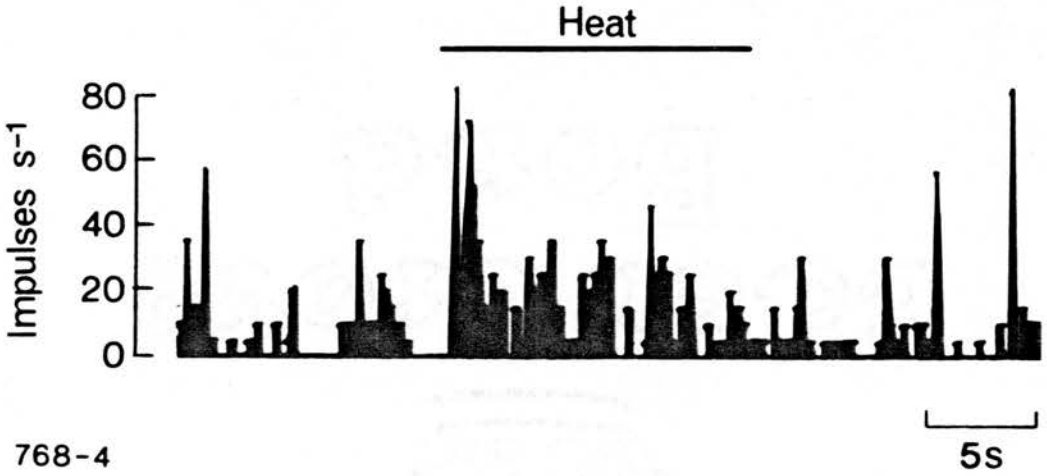

Heat
80
60
40
20
0
Impulses s-1
768-4
5s

FIGURE 2.9

Excitatory receptive fields of spinothalamic tract neurones

A : Represents the excitatory (+) and inhibitory (-) receptive fields of a multireceptive spinothalamic tract neurone. Noxious and non-noxious cutaneous stimulation on the ipsilateral thigh and forepaws was effective in producing inhibition. The unit in addition to having extensive excitatory cutaneous receptive fields also responded to flashing light into eyes. Brushing, pin-picking and pinching were the effective stimuli that produced excitation.

B : Receptive field of a specific nociceptor driven neurone. Pinch was the effective stimulus.

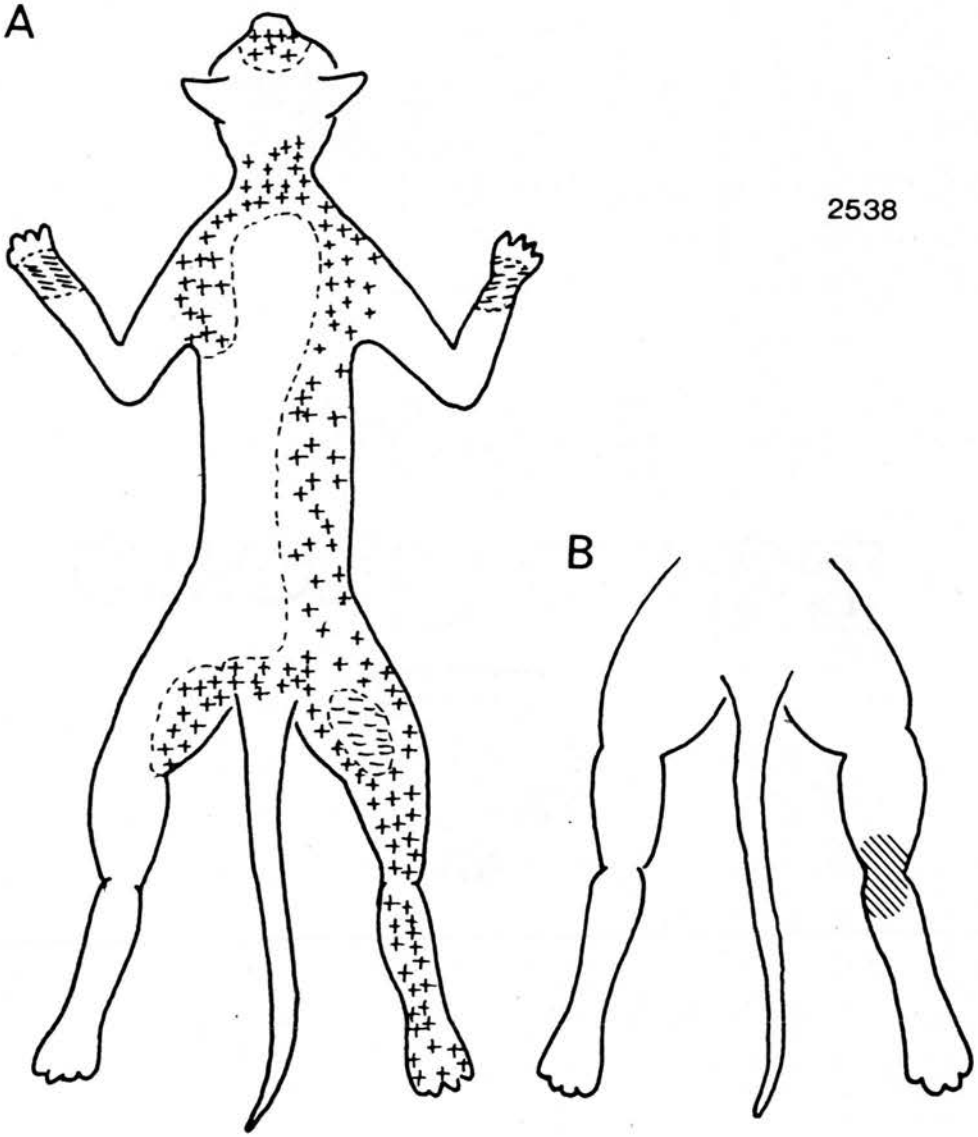
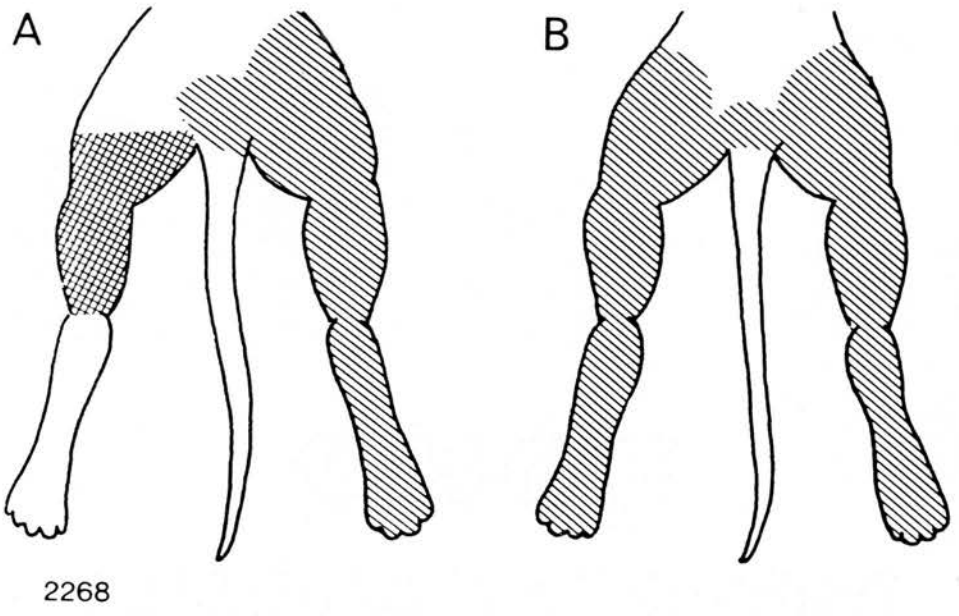

A
B
2538

FIGURE 2.10

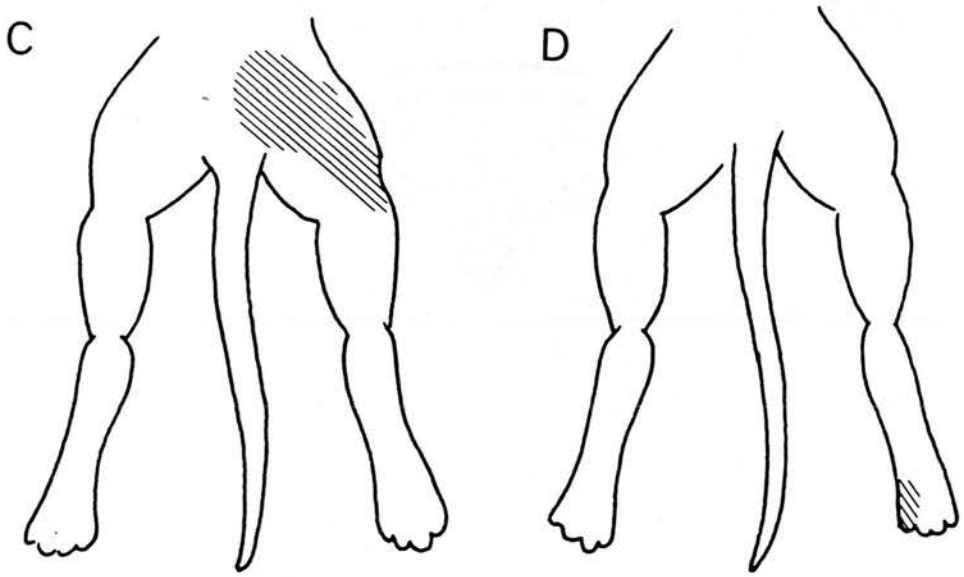
Receptive fields of neurones projecting through the contralateral VLQ or ipsilateral VLQ

A, B : Represent the receptive fields of two different neurones projecting through the contralateral VLQ. The hatched areas represent excitatory receptive fields whereas the cross-hatching represents the inhibitory field. Stroking the receptive field on the ipsilateral thigh excited the neurone whereas stroking the contralateral thigh inhibited it. Pinch applied between toes two and three also evoked responses in this cell.

C, D : Figures represent the receptive fields of two neurones projecting through the ipsilateral VLQ. Brushing was the effective stimulus in case of neurone C. Brushing, pinching and noxious heating the receptive field in case of D was effective in evoking response. The neurone having the receptive field C was located in lamina IV whereas the other (D) was in lamina I.



2268

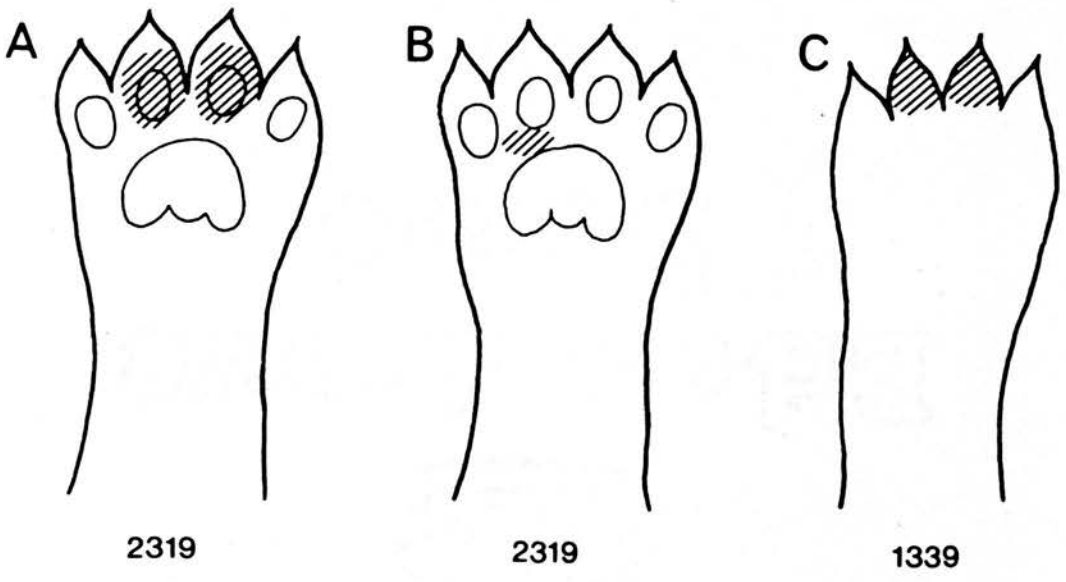


2118

FIGURE 2.11

Receptive fields of projecting lamina I specific nociceptor driven neurones

A, B, C : The excitatory receptive fields of three different lamina I specific nociceptor driven neurones are shown. Pinch applied to the receptive fields evoked the discharge in all the three neurones.


A
B
C
2319
2319
1339

SECTION III

DO ENDOGENOUS OPIOIDS MEDIATE THE INHIBITION OF SPINAL CORD NOCICEPTIVE NEURONES?

INTRODUCTION

As outlined already in a previous section on the review of the literature, there seems to be a considerable overlap in the distribution of opiate receptors, enkephalins and Substance-P in the spinal cord, all being localised in the superficial dorsal horn. The substantia gelatinosa has an equal distribution of μ and δ receptors as well as an equal number of interneurones containing Leu- and Met-enkephalin. Recently axonal flow of opiate receptors has been reported in the vagus nerve by axonal or axoplasmic transport mechanisms (Young et al. 1980). Opiate receptors have been shown to be present both in the dorsal horn and on the primary afferents, presumably on the small diameter afferents containing substance-P. Although the most common role proposed for opioid peptides (enkephalins) in the superficial dorsal horn is the modulation of transmission in the nociceptive pathways the functional role of enkephalins in this region is not fully understood.

The effect of opiates and enkephalins applied either iontophoretically in the SG or administered intravenously is a selective depression of the responses of dorsal horn neurones evoked by nocuous stimulation with little effect on the discharge evoked by innocuous stimulation (Duggan et al. 1977 a, b). The effects were reversed by the narcotic antagonist naloxone. The inhibitory effect of opiates on the activity of dorsal horn neurones responding to nocuous stimulation have also been reported in other studies (Dostrovsky and Pomeranz, 1976; Zieglgansberger and Bayerl, 1976; Belcher and Ryall, 1978). In addition to the inhibitory effect of opiates on nociceptive neurones, excitatory effects on non-nociceptive cells, antagonised by

naloxone, have also been reported (Belcher and Ryall, 1978). These studies suggest the involvement of enkephalins in the inhibitory systems impinging upon nociceptive cells or their input.

It is known that stimulation of the dorsal columns (Nashold and Friedman, 1972; Foreman et al. 1976) and contralateral peripheral nerve (Taub, 1964; Price, 1972) produce analgesia and also inhibit the dorsal horn multireceptive neurones.

This investigation was carried out to examine the role of enkephalins in the inhibition of multireceptive neurones generated by stimulation of the dorsal columns, by contralateral plantar nerve stimulation and by the tonically active descending pathways.

METHODS

Experiments were performed on nine cats (2.3 - 4.1 kg) under chloralose anaesthesia (60 mgm/kg i.v.) after induction with 4% halothane in O_2/N_2O. The skeletal neuromuscular blocker gallamine triethodide was administered intravenously to immobilise the animals. Additional anaesthetic doses were given if found necessary as the anaesthesia was monitored by allowing the animals to recover from paralysis at regular intervals, observing the pupillary diameter and by detecting any sudden increase in the arterial blood pressure. Blood pressure (arterial) was continuously monitored and all results were obtained from preparations with a minimum mean pressure of 80 mm Hg. The experimental procedures are summarised in Fig. 3.1 A.

A laminectomy was performed from L_2-L_7 and the exposed surface of the cord covered with a warm paraffin pool was maintained at 37°C by means of a feedback heating unit. Similarly the rectal temperature was continuously monitored and maintained at 37°C. A thermode was placed at L_2 for reversibly cold blocking the spinal cord as described previously (Handwerker et al. 1975; Cervero et al. 1976). A refrigerant at -5°C circulated through the thermode produced a cold block in 2-3 minutes causing a 10-20 mm Hg fall in blood pressure which returned to pre-block level after the warm fluid at 38°C was circulated through the thermode for a few minutes. Bipolar stimulating electrodes were placed on the dorsal columns at L_4 and on the left L_7 dorsal root.

Additional laminectomy in the cervical region from C_1-C_4 was performed in four cats. Bipolar silver ball electrodes were placed on the DLF at C_1 and C_3 and the dorsal columns at C_4 were sectioned. Previously described criteria were used for the identification of the spinocervical tract neurones (Brown and Franz, 1969). Accordingly units were assigned to be spinocervical tract neurones if the antidromic activity was generated from C_3 alone and not from C_1 or else the conduction velocity between C_1 and C_3 was 50% or less of the conduction velocity between C_3 and the recording site.

The hind feet were shaved and fixed to a holder, pads upwards, and the stimulating electrodes were positioned on the exposed plantar nerve of each leg. In a few experiments stimulating and recording electrodes separated by approximately 25 mm were placed on the contralateral plantar nerve for determining the threshold for the activation of Aα, Aδ and C-fibres.

Single unit recordings were made using glass microelectrodes filled with pontamine sky blue (4% in 0.5 M sodium acetate) from the L_7 segment of the spinal cord. Neurones were located having receptive fields on the left plantar surface and responding vigorously to nocuous pinch and noxious radiant heat (43-50°C for 10 secs.) as well as to non-noxious forms of stimuli. The radiant heat source described by Beck et al. (1974) was used for quantitative noxious heat stimulation of the cutaneous receptive fields. The stimulus was repeated at regular intervals of 3 minutes. Potentials were amplified and displayed in a conventional manner. Frequency histograms of the single unit activity were constructed and recorded instantaneously using a spike processor (Digitimer D130) and a chart recorder (Devices DC5H). The inhibitory effect of the dorsal columns or contralateral plantar nerve stimulation was then tested on the discharge of multireceptive or Class-2 neurones (Iggo, 1974) evoked by the noxious radiant heat.

Consistent inhibitory effects were tested further by reversibly cold blocking the spinal cord and/or administering a cumulative dose of the narcotic antagonist, naloxone. Naloxone Hcl (Endo Labs. New York) was freshly dissolved in normal saline and given intravenously (0.3 - 2 mgm/kg).

Frozen 40 µm thick transverse sections of the spinal cord, prepared in a cryostat, revealed the location of the recording sites, which were marked by the electrophoretic deposition of pontamine sky-blue with the passage of a current of 5µA for 5 minutes.

RESULTS

A total of 45 multireceptive neurones was examined in this study and 11 of these projected into the spinocervical tract. However only 14 neurones yielded satisfactory results after the administration of naloxone. Some units were lost after studying the inhibitory effect from DC and the contralateral plantar nerve but before the effect of naloxone could be tested. Reversible spinalization as previously described resulted in a few units being lost, presumably caused by sudden changes in the blood pressure. One of these cells was particularly interesting in that it showed an inhibitory effect followed by a prolonged after-discharge on application of noxious heat to its receptive field on toe-2. Noxious radiant heat applied during the period of after-discharge also inhibited the cell. Administration of 1 mgm/kg of naloxone reduced the spontaneous activity as well as the after-discharge. There was some indication of the reduction in the inhibitory effect produced by the noxious heat but nothing conclusive can be said.

The histological location of 14 neurones (2 SCT, 12 non-SCT) which yielded satisfactory results is as follows: one in lamina 1, three in lamina IV, eight in lamina V and one each in lamina VII and VIII.

Effects of naloxone on contralateral plantar inhibition (contralateral inhibition)

For producing inhibition from the contralateral plantar nerve, stimulus intensities from 1-10V, pulse width of 0.2 m sec., at 5 HZ were

employed in this investigation. At these stimulus intensities Aδ and C fibres were activated as indicated by the compound actional potential recording from the contralateral plantar nerve.

Naloxone, in doses ranging from 0.5 - 2.0 mg/kg was tested on 7 multireceptive neurones (1 SCT, 6 non-SCT) in five cats in which contralateral plantar nerve stimulation produced an inhibitory effect on the response to noxious radiant heat (44-48^{o}C) applied to the receptive field on the ipsilateral plantar surface. In the event of more than one cell being tested per animal, several hours interval was allowed between tests. Naloxone failed to produce any significant effect on five of these seven neurones with regard to the spontaneous activity, response to radiant heat or contralateral inhibition (Fig. 3.1 B).

One of the cells showed some interesting features. Weak excitation (Fig. 3.1 C) was observed in response to noxious radiant heat (48^{o}C) applied to its receptive field and this weak excitatory response changed to a strong excitation followed by prolonged inhibitory period on cold blocking the spinal cord (Fig. 3.1 C iii). The inhibitory component generated by the noxious heat was presumably under descending inhibitory control. Stimulation of the contralateral plantar nerve produced inhibition of the excitatory phase both in the intact (Fig. 3.1 C ii) and spinal state (Fig. 3.1 C iv) of the preparation. Contralateral inhibition or the inhibitory effect produced by noxious heat was not altered by the administration of naloxone (0.5 mg/kg) (Fig. 3.1 C v and vi).

Naloxone (1 mg/kg) appeared to produce a partial blockade of the contralateral inhibition on two of the seven neurones. However it was not very convincing whether it was due to the real effect on inhibition or not. In one case the spinocervical tract neurone was lost shortly after the administration of naloxone so that only one test for inhibition was performed after the drug injection. In the second neurone the inhibitory effect produced by contralateral plantar nerve stimulation caused a reduction of 74.3% in the heat response in the intact state (Fig. 3.2 a) compared to the 25.7% reduction after naloxone administration (Fig. 3.2 c). In this neurone the response to radiant heat increased by 87% after naloxone administration which may have made the cell more difficult to be inhibited (Fig. 3.2 c). This possibility is supported by the finding that eliminating the descending inhibition by cold blocking the spinal cord resulted in a vigorous discharge to the heat stimulus (Fig. 3.2 a) and a reduction or elimination of inhibition evoked by stimulation of dorsal columns or contralateral plantar nerve (Fig. 3.2 b).

Effects of naloxone on the dorsal column (DC) inhibition of multireceptive neurones

For generating the DC inhibitory effect on the noxious response of the multireceptive neurones, stimulus intensities ranging from 70-200 mV were employed. In majority of the neurones (5 of 6) the dorsal column stimulation at 10 HZ (100 μ sec. pulse width) produced a profound inhibitory effect.

The effect of naloxone (0.3 - 2.0 mg/kg) was tested on 6 dorsal horn neurones in three cats. In five neurones naloxone did not

produce any alteration in the inhibition, the noxious heat response or the background discharge of these neurones (Fig. 3.3 A). In one cell a partial reduction of 41% in the inhibition was observed (Fig. 3.4) after administration of naloxone but only one inconclusive test was performed since the cell was lost within 2 minutes after naloxone injection.

Tonic descending inhibition, effect of naloxone

In the spinalized state of the animal expanded receptive fields were observed rarely as compared to the receptive fields in the intact state. A vigorous response to noxious heat stimulus was observed in the spinal state as compared to the response in the intact state (Fig. 3.2 a, b). Expansion in receptive fields (Wall, 1967; Brown, 1971) and vigorous discharge in the spinal state (Handwerker et al. 1975) have been reported previously. Some cells responding to non-noxious forms of stimuli only in the intact state became responsive to noxious heat in the cold blocked state. In addition one specific nociceptor driven neurone in lamina I became responsive to tactile stimulation on cold blocking the spinal cord and also possessed an enlarged receptive field in the spinal state. These observations suggest the existence of tonically active powerful descending inhibitory systems.

The spinal cord was cold blocked prior to the administration of naloxone on five of the twelve occasions that it was injected and an increased response to noxious radiant heat or the background discharge in the remaining units was not altered by naloxone. Furthermore cold blocking the spinal cord following naloxone injection

revealed large increase in the response to noxious heat on four cells tested. Conversely, reversing the cold block following naloxone administration resulted in a decreased response to noxious heat on three occasions tested. Even though naloxone did cause an increase of 87% in the response to the noxious stimulus (Fig. 3.2 c) yet this increase is considerably smaller compared to the average increase of 256% in the spinal state (Fig. 3.2 b). These results clearly show the failure of naloxone to alter the tonic descending inhibition of the multireceptive neurones.

Naloxone-opiate antagonism

One experiment was conducted to check the reliability of naloxone as an opiate antagonist in these experiments since naloxone failed to influence any of the parameters measured. Morphine sulphate in two doses of 2 mg/kg given intravenously inhibited the response of a cell to noxious heat by approximately 50% (Fig. 3.3 B). The administration of naloxone six minutes later reversed the effect of morphine. Not only did this procedure result in reversal of morphine inhibition but also it caused an increased response to nocuous heat compared to the control response (Fig. 3.3 B). This increase observed was on average 130% of the control level. A transition from inhibition following morphine to excitation after naloxone has been reported by other workers (Le Bars et al. 1976; Duggan et al. 1977 a). The hypotensive effect produced on morphine administration was also reversed by naloxone.

DISCUSSION

The present study was conducted on spinal cord multireceptive or Class-2 neurones responding to noxious radiant heat and innocuous cutaneous stimulation. Thermal nociceptors in the cat hind limb are known to be innervated by C-fibres (Iggo, 1959; Beck et al. 1974; Handwerker et al. 1975). These multireceptive neurones have been suggested to be involved in the transmission of nociceptive information (Price and Dubner, 1977).

This investigation demonstrated that the response of multireceptive neurones to noxious radiant heat was not altered by naloxone except on one occasion. Even in that neurone where increase in response to nocuous heat was observed after naloxone administration it was found that the increase was considerably smaller than the one observed in the spinal state. These results confirm the finding of Duggan et al. (1977) that naloxone does not alter the tonic descending inhibition of these neurones. Under our experimental conditions endorphins do not seem to mediate the tonic descending inhibition of multireceptive neurones. Excitatory actions of opiates and opioid peptides have been reported on Renshaw cells and on neurones in the hippocampus but on neurones in the brain stem, predominantly inhibitory actions have been found (Gent and Wolstencroft, 1976; Bradley et al. 1976). However the excitatory actions of morphine, enkephalin and a new opioid dipeptide, tyrosyl-arginine (kyotorphin) have been reported on neurones in the nucleus reticularis paragigantocellularis (Satoh et al. 1979, 1980). It is still a mystery how these tonic descending pathways, possibly originating in the brain-stem, mediate their actions. Enkephalin or GABA containing interneurones in the SG could possibly

mediate these actions or perhaps some tonic descending pathways do not require the participation of spinal cord inhibitory interneurones. Predominantly inhibitory actions of enkephalins in the brain-stem suggest the occurrence of tonically active inhibitory interneurones switching off tonically active descending pathways. These pathways can be brought into action by the inhibitory effect of enkephalins on the tonically active inhibitory interneurones in the brain-stem. This assumption however raises the possibility of reduced inhibition on naloxone administration which was not observed. Though this study provides some evidence about the non-involvement of enkephalin containing interneurones in the spinal cord or known and unknown descending enkephalinergic pathways in mediating tonic descending inhibition yet this suggestion should not be strongly suggested. It is known that opiate receptors can vary in their configuration displaying differential affinity for the agonist and the antagonist. Existence of μ and δ receptors can further complicate the picture since their functional significance is not fully understood.as yet.

Morphine and D-Ala^2-MePhe^4-Met (O)-Ol^5-enkephalin display high affinity for μ receptors and antinociceptive activity whereas D-Ala^2-D-Leu^5-enkephalin have high affinity for δ receptors and possess very little antinociceptive activity (Kosterlitz, 1979; Snyder, 1980). These findings suggest the involvement of μ receptors in antinociception whereas δ receptors can play a part in regulating emotional behaviour since nucleus accumbens and olfactory tubercle, parts of the limbic system, contain high density of δ receptors. Etorphine binds uniformly to μ, δ and κ-receptors and displaces the binding of tritiated ethyl-ketazocine to κ-receptors in the guinea-pig brain whereas high concentration of μ and δ agonists are required for displacing the binding of

ethylketazocine to κ-receptor (Kosterlitz and Paterson,1980). The identification of a particular peptide and its receptor site involved in the inhibitory mechanisms is important along with the knowledge of the potency of naloxone as an antagonist on these receptor sites. It is interesting in this regard that naloxone has been reported to be a more potent antagonist of μ-receptors than on δ-receptors in the mouse vasa deferens (Kosterlitz and Paterson, 1980).

However the non-involvement of enkephalins in the tonic descending systems would not be at all surprising since the midline serotonin raphe complex has been shown to exert some tonic descending control on reflex transmission (Engberg et al. 1968 b). Above all, there could be subtle interactions between various serotoninergic, catecholaminergic or peptidergic pathways both at spinal and supraspinal levels, which can play an important part in the tonic descending inhibition. It seems important to unravel the circuitry in the dorsal horn in relation to different transmitter candidates and to study the ways enkephalin containing interneurones in the SG are modulated and how these interneurones in turn exert control over the deeper neurones. Lissauer's tract is known to contain axons of some SG neurones and on stimulation have been demonstrated to exert inhibitory control on deeper neurones and these effects closely match the inhibitory effects of morphine on these neurones (P.D. Wall, Personal Communication). These inhibitory effects were antagonised by naloxone.

Endorphins could, possibly, influence nociception by participating in the inhibitory input to nociceptive cells. Stimulation of various brain-stem and other sites produces analgesia in man and animals as well as inhibition of multireceptive neurones. These

sites include areas such as the mesencephalic periacqueductal grey (PAG) (Oliveras et al. 1974), raphe nuclei (Oliveras et al. 1975; Fields et al. 1977 a) the dorsal columns (Nashold and Friedman, 1972; Foreman et al. 1976) and contralateral peripheral nerve (Taub, 1964; Price, 1972). However recently reported findings indicate the failure of naloxone to antagonise the descending effects from the medullary raphe (Duggan and Griersmith, 1979) and mesencephalic PAG (Carstens et al. 1979 b). It is interesting that our findings too provide evidence that naloxone does not antagonise the inhibition of these neurones from dorsal columns and the contralateral plantar nerve.

The inhibition from the contralateral plantar nerve stimulation could be evoked only when Aδ and C-Fibres were activated whereas low stimulus intensities were sufficient for the dorsal column inhibition. Neurones inhibited by contralateral plantar stimulation were also inhibited by noxious pinch applied on the contralateral plantar surface whereas non-noxious forms of stimuli did not produce any inhibition. This contralateral plantar inhibition would appear similar in some respects to a recent study by Le Bars et al. (1979) but these authors could not demonstrate inhibition in the spinal state whereas this study could. Absence of contralateral inhibition in the spinal state was suggested to be due to the participation of supraspinal structures. Our study compared the contralateral inhibition of 10 cells before and during the cold block of the spinal cord. It was observed that the inhibition was less in the spinal than in the intact state and in two neurones it almost disappeared during cold block. However the increased discharge of the neurone to noxious radiant heat during the spinal state could have masked the inhibition. In addition, Hongo et al. (1968) have demonstrated poly-

synaptic inhibitory postsynaptic potentials from high threshold muscle and cutaneous afferents of the contralateral hind limb in spinal cats.

The evidence presented would seem to exclude the possibility of the involvement of endorphins (Endogenous opioid peptides) in the types of inhibition examined but it does not rule out the modulatory role that endorphins might play in nociception. However other factors could play a part in the results obtained. Pert and Snyder (1974) have demonstrated the existence of opiate receptor in two configurations, one displaying high affinity for the agonist and the other for the antagonist. And the relative proportion of these two configurations can vary. It is quite possible that the use of anaesthetized animals precludes the activation of an endorphin system. The part played by endorphins in the modulation of nociception by stress (Madden et al. 1977) would not be revealed in the anaesthetized animal. It is interesting, in this regard, that almost all investigations of nociception where naloxone was found to exert an effect were carried out on unanaesthetized subjects (Jacob et al. 1974; Buchsbaum et al. 1977; Mayer et al. 1977). However there are reports where naloxone did not exert any effect (Goldstein et al. 1976; El-Sobky et al. 1976; Grovert and Goldstein, 1978). The effectiveness of naloxone to influence may well be determined by several factors such as the preparation used, the time of the test and whether the test is a measure of the nociceptive threshold or tolerance (Frederickson, 1978).

FIGURE 3.1

A. Shows diagrammatically the experiment arrangement. DLF = dorsolateral funiculus; DC = dorsal columns; DR = dorsal roots; c. plantar n. = contralateral plantar nerve; i. plantar n. = ipsilateral plantar nerve. C_1, C_3, C_4 = cervical segments 1, 3 and 4. L_2, L_4, L_7 = lumbar segments 2, 4 and 7.

Effect of naloxone on contralateral plantar inhibition

B. Frequency histograms of the action potentials of a Class-2 neurone showing from left to right: before 0.5 mg kg^{-1} naloxone i.v. - response to raising temperature of cutaneous receptive field on the ipsilateral hind paw to 48°C for 10s followed by response to an identical heat stimulus 5 mins. later during electrical stimulation of the contralateral plantar nerve (5 HZ, 0.2 ms pulse duration, at intensity sufficient to excite all A fibres); after i.v. injection of naloxone - responses of cell to a series of stimuli identical to that above. Time scale as in C.

C. From left to right are shown the responses of another Class-2 neurone to a series of stimuli identical to those in A, before cold-blocking the spinal cord rostral to a recording site (i and ii), during cold-block (iii and iv) and finally after injection of naloxone (1.5 mg kg^{-1} i.v.) during cold-block (v and vi).

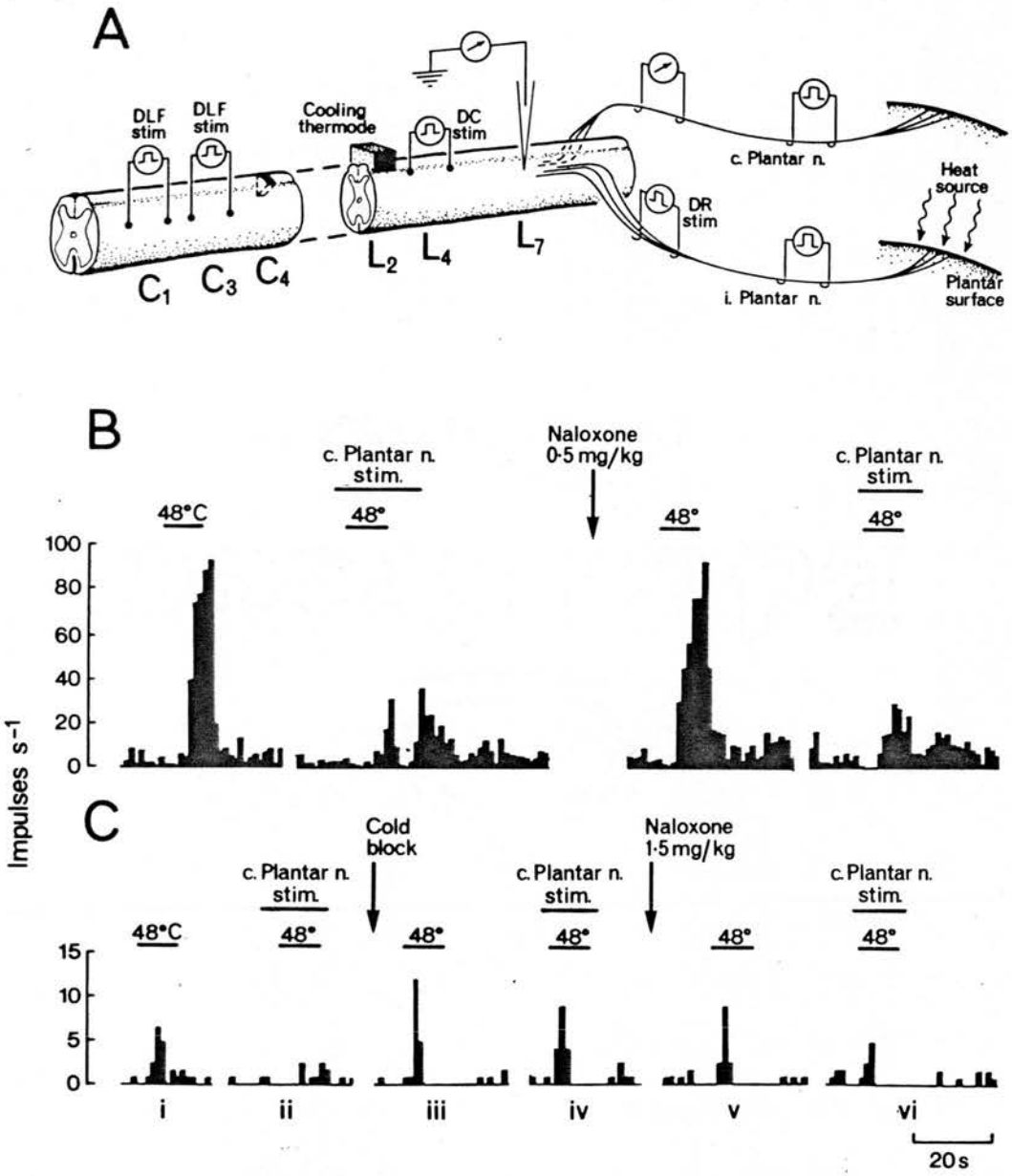

A
DLF stim
DLF stim
Cooling thermode
DC stim
c. Plantar n.
Heat source
DR stim
C1
C3
C4
L2
L4
L7
i. Plantar n.
Plantar surface
B
c. Plantar n. stim.
Naloxone 0·5 mg/kg
c. Plantar n. stim.
48°C
48°
48°
48°
Impulses s-1
100
80
60
40
20
0
C
Cold block
Naloxone 1·5 mg/kg
c. Plantar n. stim.
c. Plantar n. stim.
c. Plantar n. stim.
48°C
48°
48°
48°
48°
48°
15
10
5
0
i
ii
iii
iv
v
vi
20 s

FIGURE 3.2

Frequency histograms of the response of a lamina V multi-receptive neurone evoked by heat stimulus at the 47°C applied to the cutaneous receptive field for 10 seconds. The lower thin bars represent the heat stimulus at 47°C and upper thick bars represent the contralateral plantar stimulation at 2 V, 5 HZ, 200 μ sec. pulse width. Time scale as in c.

a) From left to right: before the cold block, control response to 47°C followed by the response to an identical stimulus applied during contralateral plantar nerve stimulation followed three minutes later by the control response. After cold-block the same heat stimulus evoked a greatly increased discharge.

b) Same neurone as in a) showing increased discharge to heat stimulus during spinal state and the inability of the contralateral plantar nerve stimulation to produce any obvious inhibitory effect as shown in a).

c) From left to right: after reversal of the spinal state the response of the same neurone as in a) and b) to noxious heat at 47°C followed by the response to an identical stimulus after intravenous administration of naloxone (1 mg/kg). This increased control response is followed by the response to the same stimulus during the contralateral plantar stimulation.

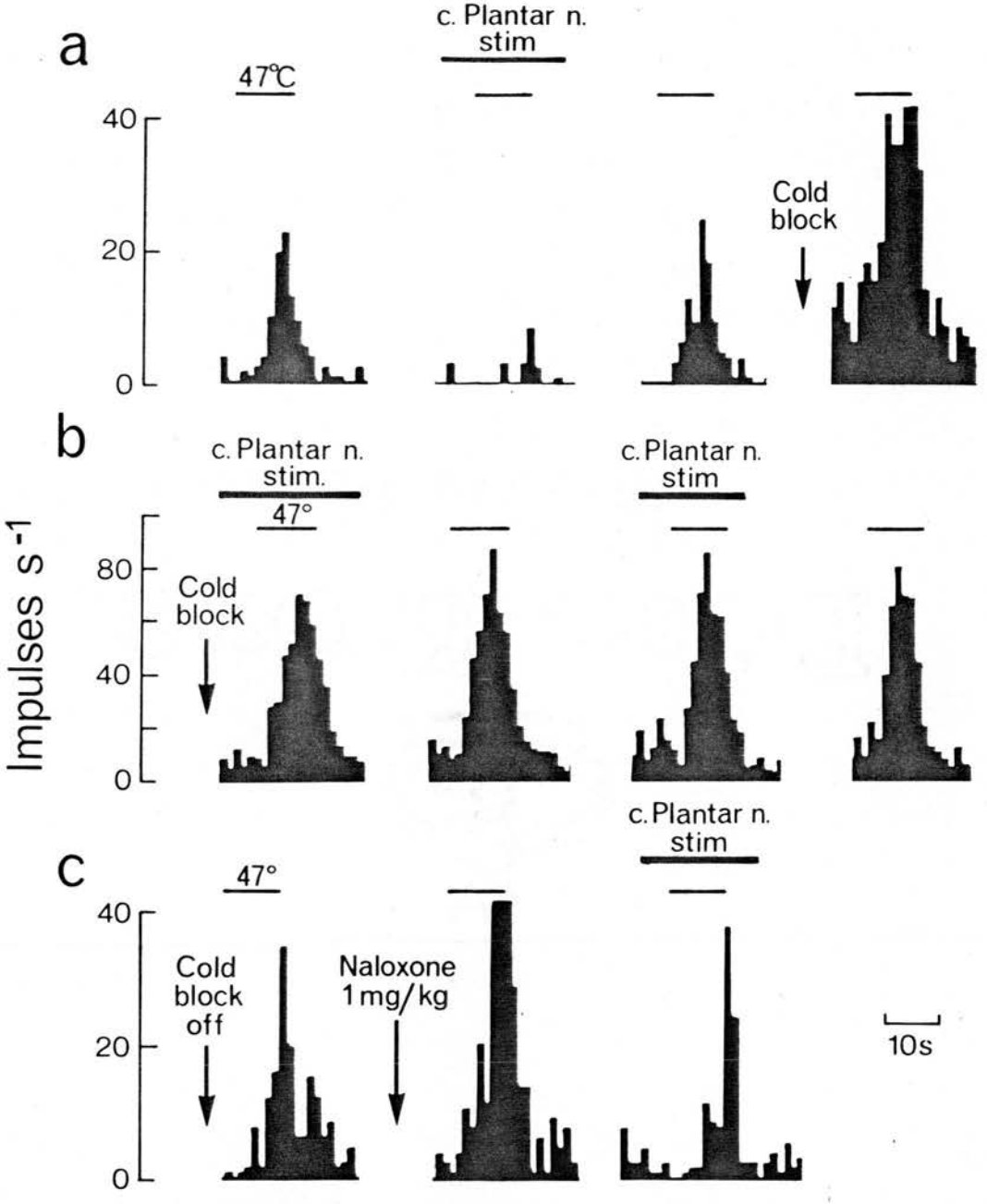

a
c. Plantar n. stim
47°C
40
20
0
Cold block
b
c. Plantar n. stim.
47°
c. Plantar n. stim
80
40
0
Cold block
Impulses s-1
c
c. Plantar n. stim
47°
40
20
0
Cold block off
Naloxone 1 mg/kg
10s

FIGURE 3.3

A. Effect of naloxone on the dorsal column inhibition

Frequency histograms of the action potentials of a Class-2 neurones showing, from left to right - its response to raising the temperature of its cutaneous receptive field on the ipsilateral hind paw to 44°C for 10s, response to identical heat stimulus 5 minutes later during electrical stimulation of the ipsilateral dorsal columns (10 HZ, 0.2 ms pulse duration, 100 mV) rostral to the recording site followed by a further response to the heat stimulus alone; after injection of naloxone 2 mg kg^{-1} i.v. responses of the cell are shown to a series of stimuli identical to that described above indicate no change in the dorsal column inhibition.

B. Naloxone, antagonism of morphine action

Frequency histograms of the responses of a Class-2 neurone to noxious heating (horizontal bars) of its receptive field before morphine 4 mg kg^{-1} i.v. (left), after morphine (centre) and subsequently after naloxone 0.1 mg kg^{-1} i.v. (right).

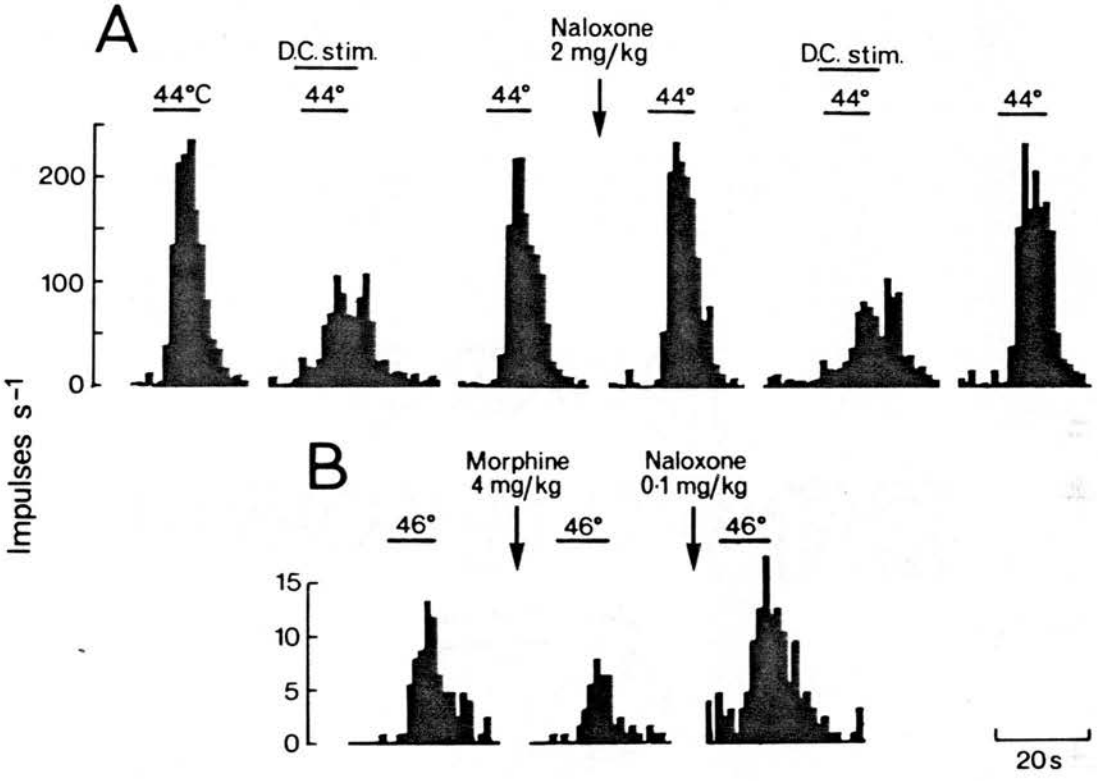
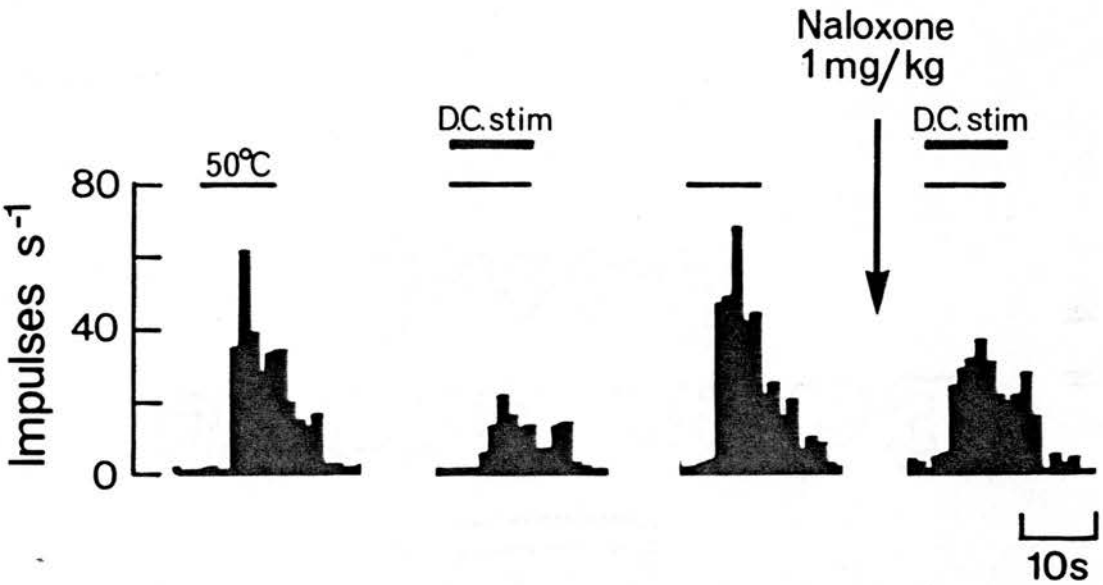

A
D.C. stim.
Naloxone
2 mg/kg
D.C. stim.
44°C
44°
44°
44°
44°
44°
200
100
0
Impulses s⁻¹
B
Morphine
4 mg/kg
Naloxone
0·1 mg/kg
46°
46°
46°
15
10
5
0
20 s

FIGURE 3.4

Effect of naloxone on the dorsal column inhibition

Frequency histograms of the response of a Class-2 neurone to a heat stimulus at 50°C applied for 10 seconds. The lower thin bars represent the heat stimulus and the upper thick bars represent the dorsal column stimulation (200 mV, 10 HZ, 100 μ sec. pulse width).

From left to right: response to 50°C heat followed by the response to an identical stimulus in the presence of the dorsal stimulation, showing inhibition. Control response is repeated after this followed by the response to the same nocuous heat stimulus (50°C) in the presence of DC stimulation after the administration of naloxone (1 mg/kg).


Naloxone
1 mg/kg
D.C. stim
D.C. stim
50°C
80
40
0
Impulses s-1
10s

SECTION IV

DESCENDING CONTROL OF SPINAL NOCICEPTIVE TRANSMISSION FROM THE NUCLEUS LOCUS COERULEUS

INTRODUCTION

A brief introduction will be given here since a detailed survey of the relevant literature has been presented in Section I. The nucleus locus coeruleus (LC) apart from containing catecholamine somata has also been recently demonstrated to contain substance-P and neurotensin. Nerve terminals containing NA, 5-HT, DA, substance-P, Met-enkephalin, β-endorphin and Ach etc. are known to be present in LC. The axon terminals showing substance-P and Met-enkephalin-like immuno-reactivity in the locus coeruleus have been reported to be morphologically identical and make asymmetric axo-dendritic synapses. These axon terminals were shown to be similar to the type which established asymmetric axo-dendritic synapses with dendrites labelled for the enzyme tyrosine-β-hydroxylase in LC (Pickel et al. 1979). However it has been suggested previously that Met-enkephalin containing interneurones in the spinal cord establish axo-axonic contacts with the substance-P containing primary afferent terminals (Hökfelt et al. 1977a). The axon terminals showing substance-P and Met-enkephalin immunoreactivity in LC contained a few large dense vesicles (LDVs) and these have been suggested to be the primary storage site for peptides (Pickel et al. 1979).

The nucleus locus coeruleus (LC) is reported to be involved in such diverse functions as analgesia, reward, sleep, motivation and micturition etc. The small population of neurones in this nucleus innervate wide areas of the central nervous system thus providing the anatomical substrate for various physiological functions. The electrical stimulation or microinjection of morphine into LC produces behavioural analgesia and the analgesia produced by the systemic

administration of morphine is abolished on lesioning the nucleus locus coeruleus. A coeruleospinal pathway has been described in the rat, cat, monkey and man. The nucleus locus coeruleus projects widely in the CNS including the nucleus raphe magnus (NRM) which is also known to project to the spinal cord.

In a recent elegant study in the monkey, Westlund and Coulter (1980), using a combination of retrograde transport of HRP, anterograde autoradiography and dopamine-β-hydroxylase immunocytochemistry, have provided some important information on the origin, course, terminations and the nature of the chemical transmitter in descending fibres from the nucleus locus coeruleus. The terminations in the spinal cord were found ipsilaterally in laminae I, II, IV-VI, VII-IX and X. Based on the dopamine-β-hydroxylase immunocytochemistry, noradrenaline has been suggested to be the transmitter contained in descending fibres from LC. The bilateral spinal terminations from the subcoeruleus (SC)-medial parabrachialis (PB) complex have also been demonstrated. Microiontophoretic studies suggest the inhibitory effect of NA on spinal cord neurones (Engberg and Ryall, 1966; Headley et al. 1978). A selective inhibitory action on the nociceptive component of multireceptive neurones has also been observed by the microiontophoretic application of NA and 5-HT in either the SG (Headley et al. 1978) or closer to the cell bodies of these deeper neurones (Belcher et al. 1978).

This investigation was carried out in the light of the evidence presented to study the following:

(A) (i) Descending influences from the nucleus locus coeruleus on the spinal cord nociceptive transmission.

(ii) Do the nucleus locus coeruleus (LC) and the nucleus raphe magnus (NRM) exert similar actions on the discharge of the same multireceptive neurones or not?

(B) The involvement of putative transmitters in mediating descending effects from LC and NRM.

(C) The contribution of direct coeruleospinal as well as indirect pathways through other brain-stem nuclei such as the nucleus raphe magnus (NRM) in mediating the locus coeruleus modulation of the spinal sensory transmission.

(D) The contribution of pre- and postsynaptic mechanisms underlying the modulation of spinal sensory transmission from the nucleus locus coeruleus (LC).

METHODS

Experiments were performed on 48 anaesthetized and paralysed cats weighing 2.5 - 3.5 Kg. The surgical, recording, data collection and histological procedures were essentially the same as described for experiments of Sections II and III. Glass microelectrodes filled with pontamine sky-blue were employed for recording the single unit

activity of neurones in the lumbar spinal cord. The recordings in the majority of the cases were made from multi-receptive neurones that receive both noxious and non-noxious cutaneous input. Occasionally recordings were also made from the mechanoreceptive and specific nociceptor driven (nocireceptive) neurones. The natural cutaneous stimuli were applied as described in sections II and III. The effect of LC stimulation was also tested on the discharge evoked by electrical stimulation of the ipsilateral tibial nerve. The bipolar stimulating and recording electrodes were placed 20 mm apart on the ipsilateral tibial nerve. This allowed the thresholds for the activation of Aα, Aδ and C fibres to be determined by monitoring the compound action potential.

Potentials elicited by the natural cutaneous and electrical stimulation were amplified and displayed using conventional means and were recorded on a FM data tape. These amplified action potentials were also led to a spike processor (Digitimer D130) and a chart recorder (Devices DC 5H) which enabled construction of instantaneous frequency histograms of the activity which was subsequently tested further for effects produced by the electrical stimulation in the nuclei locus coeruleus (LC) and raphe magnus (NRM). The output of the spike processor was continuously monitored on an oscilloscope and the level of the spike discrimination adjusted accordingly to ensure recordings from the same single unit under investigation. In the majority of recordings, only the lower level of the gate was used but occasionally the window discriminator was also used in order to discriminate the unit from a multiunit recording. In the later stages of the investigation the 'dot raster display' technique as described

by Mendell (1966) was also employed for the data display. A saw-tooth having a short period as compared to the horizontal deflection time was applied to the vertical deflection plates so that the lines were almost vertical. The amplified action potentials were used to trigger the brightening pulses which were fed onto the Z axis of the storage oscilloscope. The intensity of the storage oscilloscope was turned down to a level that allowed only the brightening pulses to be seen as dots, which were stored and photographed.

A left craniotomy was performed for placing the stimulating electrodes into LC and NRM. Parts of the tentorium were carefully removed which allowed an electrode to be placed vertically in NRM at the stereotaxic co-ordinates P8, H-8, LO, in the majority of experiments. However in some experiments electrodes were also placed at P6, 7 and 9. The concentric bipolar stainless steel electrodes described in Section II were used for stimulating NRM and LC. The nucleus locus coeruleus (LC) was approached caudally at an angle of 45° to the horizontal plane so as to avoid the tentorium. An array of stimulating electrodes similar to the one used in Section II was also employed in this part of the investigation for stimulating LC. According to the atlas of Berman (1968) the electrodes were aimed at P2-4, L2.0-3.5, H-2 → -2.5 in the majority of experiments. This was also aided by various publications related to the locus coeruleus which are mentioned later in the section on the histological reconstruction of the stimulation sites. However in a few experiments the most rostral parts of the nucleus were also stimulated using a single electrode. In the initial experiments, cerebellum was removed either before or at the end of the experiment to allow for the visual exami-

examination of the midline and the dorsal surface of the brain-stem. This provided some information on the accuracy of placing electrodes stereotaxically which was subsequently verified histologically in each experiment. The stimulation of LC has been reported to produce bladder contractions (Lalley et al. 1972; Amaral and Sinnamon, 1977) and some attention was paid to this aspect for placing electrodes in the nucleus locus coeruleus (LC).

In order to investigate part C of the present study, lesions were made in NRM and in the spinal cord at the lumbar and thoracic levels. The midline raphe lesions that included NRM were made by passing a DC current of 300 μA for 20-30 seconds. The lesions in the midline raphe region were placed at the stereotaxic co-ordinates P5-13, H-3 → -10, L=0. At each posterior co-ordinate 7 or 8 lesions spaced 1 mm apart were made thus effectively covering the dorso-ventral extent (-3 → -10). Some of the lesions were placed stereo-taxically whereas the others were made by visual examination of the dorsal surface of the brain-stem after removing the cerebellum. At the most rostral and caudal end of the lesions, 1 mm laterally placed lesions were also made in one experiment. The spinal cord lesions were made by using a fine blade, a pair of spring scissors or fine forceps and included various quadrants of the cord at the lumbar and thoracic levels. These brain-stem and spinal cord lesions were reconstructed histologically.

The drug solutions were freshly made and administered intra-venously through a cannula in the femoral vein. The serotonin

antagonists methysergide hydrogenmaleinate (Sandoz Ltd. Basle) and Cinnanserin HCl (Squibb) were freshly dissolved in normal saline. The noradrenergic α-blocker, phenoxybenzamine (SKF labs. Ltd.) was dissolved in an acidified mixture of equal volumes of ethanol and propylene glycol and diluted with normal saline. The noradrenergic α-blockers, yohimbine hydrochloride (Aldrich), and β-blockers, sotalol hydrochloride (Bristol Labs.), propranolol hydrochloride, (ICI Ltd.) were also administered intravenously. The opiate antagonist naloxone hydrochloride (Endo Labs., New York) was freshly dissolved in normal saline and the GABA antagonist bicuculline (Sigma) was made soluble by warming and acidification with 0.1 N HCl.

At the end of each experiment, 1-3 mA DC current was passed through the stimulating electrodes for the histological location of the sites stimulated in LC and NRM. The animals were perfused with 150 - 200 ml of formal-saline given through the femoral artery. The recorded sites marked with pontamine sky-blue as well as the extent of various surgical lesions were reconstructed from 40 μm thick transverse sections of the spinal cord. The brain-stem was left in formal saline for five days before cutting the transverse sections. The lesions made in the NRM and LC were reconstructed from 50 μm thick serial, transverse sections of the brain-stem stained with cresyl violet. These cresyl-violet stained sections were examined under a light microscope (Zeiss or Nikon) and reconstructions were made by using a camera lucida attachment (Zeiss) or a similar drawing tube (Sankei) attached to the Nikon light microscope. The position of each site of stimulation was studied, determined and reconstructed from the cresyl violet stained serial transverse sections with the

help of the atlas of Berman (1968). The histological location of stimulation sites was further aided by various publications on the nucleus raphe magnus and locus coeruleus (Taber et al. 1960; Taber, 1961; Chu and Bloom, 1974; Kuypers and Maisky, 1975, 1977; Hancock and Fougerousse, 1976; Tohyama et al. 1979; Sakai et al. 1979).

RESULTS

PART A

(i) Descending influences from the nucleus locus coeruleus on the spinal cord nociceptive transmission.

(ii) Do the locus coeruleus (LC) and nucleus raphe magnus (NRM) exert similar actions on the discharge of the same multireceptive neurones or not?

(i) Descending influences from the nucleus locus coeruleus (LC) on the spinal cord nociceptive transmission

The effects of locus coeruleus stimulation were studied on the discharge of multireceptive neurones that respond to noxious and non-noxious cutaneous stimulation. A total of 132 multireceptive neurones was tested for modulation from the locus coeruleus in 48 cats in which the subsequent histological examination revealed the location of the stimulation site within the boundaries of the nucleus locus coeruleus. The effect of locus coeruleus stimulation was also investigated on the activity of specific nociceptor-driven as well as on the discharge of neurones excited only by non-noxious cutaneous stimulation. The effect of LC stimulation was studied on the spontaneous discharge (background discharge) and also on the discharge evoked by natural cutaneous and/or electrical stimulation of the tibial nerve.

Locus coeruleus stimulation produced an inhibition of the discharge of 104 multireceptive neurones. There was a mixed early excitation followed by a period of later inhibition on 18 of these neurones. The excitatory action alone on the activity of 8 multireceptive neurones have also been observed and two of these neurones received input from the joint and muscle in addition to the cutaneous input. So far, in this sample, only two multireceptive neurones were observed on which LC did not exert any action.

Effects of LC stimulation on the background discharge of multireceptive neurones

The majority of the multireceptive neurones showed background discharge which was under a strong modulatory influence from the locus coeruleus (LC) (Figs. 4.1, 4.2). Both the inhibitory (Figs. 4.1 A, 4.2 A) and excitatory (Fig. 4.2 C) actions were observed from the locus coeruleus stimulation. However the inhibitory actions produced by the LC stimulation were predominant as has been pointed out in the beginning. The recruitment of the inhibition was also studied which revealed that increasing the current intensity (Figs. 4.1 D, 4.2 B), the train length (Fig. 4.1 C) or the frequency of stimulation (Fig. 4.1 B) produced an increased inhibition from LC on the background or the evoked discharge (Fig. 4.7 a).

Inhibition of the nociceptive input of multireceptive neurones by LC stimulation

The modulatory influence of LC stimulation was studied on the discharge of multireceptive neurones evoked by noxious cutaneous stimulation such as pinch or noxious radiant heat (43-53^{o}C) applied for 10 seconds on the cutaneous receptive field of the unit. There was a strong inhibition of the response to pinch (Fig. 4.3 A) or heat (Fig. 4.3 B) on electrical stimulation in the nucleus locus coeruleus. The noxious cutaneous stimuli were applied at regular intervals of 3-5 minutes. The multireceptive neurones showed a stepwise rise in their discharge on increasing the level of noxious

heat applied on the cutaneous receptive field (Figs. 4.4, 4.5) and when tested in the presence of LC stimulation decreased the heat discharge producing a parallel shift in the heat response (Fig. 4.5).

Recruitment of inhibition on the discharge evoked by noxious stimuli

Increasing the current intensity (Fig. 4.7 a) or the repetition rate of trains of stimuli (Fig. 4.7 C) revealed the recruitment of inhibition from the locus coeruleus on the heat evoked discharge of multireceptive neurones. As it is shown in the various figures mentioned above, inhibition from 20-80% in the heat or the pinch evoked discharge was seen, depending on the stimulation parameters used. However occasionally a complete inhibition of the discharge evoked by the noxious heat was also observed from LC.

Time course of the inhibitory action from LC on the discharge evoked by the noxious heat stimulus

The inhibition of the noxious heat response was observed during the whole period of stimulation of the locus coeruleus (Fig. 4.6). The time course of the inhibitory action on the evoked response to heat applied for 10 seconds at various intervals before, during and after the locus coeruleus stimulation was studied on 2 multireceptive neurones. The inhibition starts when the heat stimulus is applied 5 seconds before the onset of LC (Fig. 4.6). Strong inhibition of the heat response was seen when the heat stimulus was applied concurrently with the onset of LC. A similar magnitude of inhibition was observed thereafter on applying the heat stimulus at various

intervals after the onset of LC stimulation. However no inhibition was observed if the heat stimulus was applied 5 seconds before the LC was turned off which indicates that the inhibitory effect of LC stimulation overlasts the period of stimulation for a period of less than 10 seconds. There was no postinhibitory rebound seen in this particular neurone (Fig. 4.6) but it was observed on several other neurones. However it was not as frequent as the rebound seen after the nucleus raphe magnus stimulation.

The inhibitory action from LC on the noxious versus the non-noxious response of multireceptive neurones

The inhibitory action of the locus coeruleus stimulation has been compared on the discharge of multireceptive neurones evoked by the noxious versus the non-noxious cutaneous stimulation. A selective reduction in the discharge evoked by noxious stimuli was observed on 50% of the neurones examined (5 of 10). In these five units LC stimulation did not produce inhibition or excitation of the discharge evoked by non-noxious cutaneous stimuli. However in the remaining five units there was a non-selective reduction in the discharge evoked by both the noxious and the non-noxious cutaneous stimulation. It was however observed that even though there was an inhibition of the discharge evoked by both the noxious and non-noxious cutaneous inputs the inhibitory action was much more predominant on the nociceptive input than on the non-nociceptive input (Fig. 4.7 a). There was a reduction of 70% in the nociceptive evoked discharge compared to a 25% reduction in the non-nociceptive discharge. However at least on two of these units the magnitude of inhibition was similar on both

inputs. The electrical stimulation in LC also revealed a strong inhibition of the Aδ and C-fibre evoked discharge but the early A fibre evoked discharge was also seen to be reduced to a lesser degree as compared to the reduction in the latter part of the discharge produced by Aδ and C-fibres.

Postinhibitory rebound and wind-up of inhibition from LC and NRM

Postinhibitory rebound was observed on some multireceptive neurones inhibited by LC stimulation. The occurrence of the post-inhibitory rebound was not as frequent from LC as it was from the nucleus raphe magnus (NRM). However on some cells postinhibitory rebound of a similar magnitude was observed from LC and NRM.

There has been only one instance so far where a wind-up of the inhibition was seen from both LC and NRM stimulation (Fig. 4.20 a).

Effect of LC on the discharge evoked by the electrical stimulation of the tibial nerve

The effect of LC stimulation was investigated on the discharge evoked by electrical stimulation of the tibial nerve. It has been described in the methods section that the compound action potential was monitored by placing the stimulating and recording electrodes on the tibial nerve to find the threshold for the activation of Aα, Aδ and C-fibres. On the activity evoked by the electrical stimulation of the ipsilateral tibial nerve and the dorsal root it was observed that the inhibitory effect of LC and NRM stimulation was

more effective on the discharge evoked by the Aδ and C fibres but nevertheless it also reduced the discharge evoked by the other components of the A-fibre (Figs. 4.8 a, 4.16 a, 4.18 a, 4.22 A, 4.23 A, 4.25 a, b).

Mixed excitation and inhibition from LC on multireceptive neurones and the duration of inhibition

As has already been described briefly that a mixed excitation and inhibition produced by LC stimulation have also been observed on 18 multireceptive neurones. The excitation usually had a shorter latency and was subsequently followed by a period of inhibition lasting longer than the excitation (Fig. 4.23 B). Rarely a later period of excitation after the inhibition was also observed (Fig. 4.23 B). The excitation from LC lasted for up to 100 m sec. only whereas the subsequent inhibition has been seen to last longer than 1 second on some neurones. Varying the interval between the conditioning and test stimuli as well as testing the inhibitory effect of LC on the spontaneous discharge revealed that the inhibition lasts for as long as 1 second (Fig. 4.8 d).

Effect of the locus coeruleus stimulation on the discharge of neurones excitated by non-noxious cutaneous stimulation only (mechanoreceptive neurones or Class 1)

Locus coeruleus (LC) stimulation was tested on the activity evoked only by the non-noxious cutaneous stimulation of 10 mechanoreceptive neurones. The discharge evoked by the natural innocuous cutaneous stimulation did not seem to be under any modulatory

influence from LC on six of these neurones (Fig. 4.9). However inhibition of the discharge evoked by the non-noxious cutaneous input was observed from LC stimulation on three neurones. Locus coeruleus stimulation also caused an excitation on one of the mechanoreceptive neurones.

(ii) Effect of stimulation of locus coeruleus and nucleus raphe magnus on the activity of the same multireceptive neurones

The effects of LC stimulation were tested and compared with the actions exerted by NRM on the activity of the same multireceptive neurone. The stimulation of LC and NRM was tested on the spontaneous as well as the evoked activity of 92 multireceptive neurones in order to find similarities or dissimilarities between the actions from these two nuclei. This was conducted in order to find if a single unit was inhibited or excited from only one nucleus or from both which might provide some indication on the pathways involved in mediating the descending actions from LC and NRM. Out of the total 92 neurones examined, inhibition from the brain-stem was found on 69 of these units. The inhibitory action was observed on 67 neurones from both LC and NRM (Fig. 4.1 D, E; 4.3 B; 4.7 a, b; 4.8; 4.16-4.20) whereas the remaining two units showed inhibition from only the NRM. The mixed effects of early excitation followed by a period of inhibition lasting longer than the excitation was found on 20 neurones. The initial excitation lasted for up to 100 m sec whereas the subsequent inhibition lasted up to one second or more (Fig. 4.8 d). The nucleus locus coeruleus produced an inhibitory effect on four of these neurones whereas NRM exerted an early excitation followed by a

period of inhibition (Fig. 4.8 d). Three of these neurones were excited and then inhibited by LC stimulation but were only inhibited by NRM (Fig. 4.8 C). Three units were excited by the brain-stem and one of these was excited from NRM alone. Although in a majority of neurones, LC and NRM evoked-inhibition was similar (Fig. 4.8 b, d; 4.23 A) but rarely inhibition greater from LC (Fig. 4.8 a) or from NRM (Figs. 4.3 b; 4.7 b; 4.8 C) was also observed. In a few multireceptive neurones when dissimilar inhibition was observed from LC and NRM, the nucleus raphe magnus was observed to exert stronger inhibition as compared to LC.

Spinal cord location of the multireceptive neurones investigated (Parts A-D)

The location of the majority of the multireceptive neurones is shown in Fig. 4.10. As is evident from the figures the multireceptive neurones inhibited, or excited and inhibited were scattered all over the dorsal horn. These multireceptive neurones whether located in lamina I or V are inhibited by LC and NRM stimulation. Similarly in some of the units recorded in the substantia gelatinosa which were observed to display similar characteristics to the other dorsal horn neurones examined, both inhibitory (3 neurones), as well as mixed excitatory and inhibitory effects were observed (2 neurones) from LC and NRM. However in the lamina I region inhibitory effects have been observed from both LC and NRM on all multireceptive neurones excepting one neurone which showed mixed effects to LC and NRM stimulation. A few specific nociceptor driven neurones tested in this region (3 neurones) also showed inhibitory effects produced from

both LC and NRM stimulation. Some of the deeper units in laminae VI and VII which in addition to cutaneous inputs also received muscle and joint inputs showed a strong excitatory effect produced by LC and NRM stimulation.

The Effect of LC stimulation on micturition and blood pressure

The stimulation of the nucleus locus coeruleus caused urine flow and also produced a small transient pressor response. The stimulation in LC causing contraction of the urinary bladder as well as a pressor response is already known (Amaral and Sinnamon, 1977).

Stimulation parameters and thresholds from LC

For producing the inhibitory action from LC various stimulus intensities ranging from 50-300 μA were employed. The threshold for the inhibitory effects varied from 50-130 μA. A train of 60-100 m sec with an interpulse interval of 2-5 m sec (200-500 HZ) was repeated at 0.5-2 HZ. The square wave pulses in the train had a pulse width of 200 μ sec. However even shorter trains were effective in producing inhibition as shown in Fig. 4.1 C. In a majority of the experiments a train of 60 m sec (200 HZ) was repeated once every second. However for the excitatory effects the thresholds were smaller (<50 μA). The conduction velocity calculated on the basis of the latency of the first spike evoked from LC, ranged from 8-70 m/sec.

Stimulation parameters and thresholds from NRM

For studying the effect of NRM stimulation, intensities varying from 50 μA to 300 μA were used. A train (60-80 m sec) having an interpulse interval of 2-5 m sec (200-500 HZ) and a pulse width of 200 μ sec was repeated at 0.5-2 HZ. In the majority of experiments the train length (60 m sec) at 200 HZ was repeated once every second. The thresholds for producing inhibition varied from 50-100 μA. Even the shorter trains (10-50 m sec) were effective in producing the inhibitory effect.

Location of LC stimulation sites
(Parts A-D)

The stimulation sites were studied, determined and reconstructed from the cresyl-violet stained serial transverse sections of the brain-stem as has been described in the methods section. The rostrocaudal extent of the nucleus from P2-4 was investigated in the majority of experiments. The stimulation sites (Fig. 4.11, P2-4) are represented in composite pictures showing the majority of the stimulation sites in the regions of the nucleus locus coeruleus stimulated in the present investigation (Parts A-D). The effects reported in the investigation (Parts A-D) on the multireceptive neurones were produced from these stimulation sites (Fig. 4.11) which were placed either within or directly adjacent to the nucleus locus coeruleus (LC). The filled circles represent sites producing an inhibition whereas the open circles represent areas producing an excitation. The rostral part of the nucleus at P1 was also stimulated in two experiments which

also produced effects similar to the actions produced from other parts of the nucleus. However the stimulation at this most rostral site was abandoned since at this most rostral site the nucleus is embedded in the ventrolateral part of the periaqueductal gray. In order to obtain some indication of the current spread, the medial electrode of the pair was placed such that it was approximately 1 mm outside the nucleus and the lateral electrode within the nucleus locus coeruleus. Four such experiments as these are illustrated in Fig. 4.11 (P2, P3) and it was observed that the maximum current intensity of 300 μA used in this study did not produce any effect (Fig. 4.1 F) from the medial electrode in the pair represented by open triangles whereas the lateral electrode represented by filled triangles did modulate the activity of the multireceptive neurones under investigation (Fig. 4.1 A). This observation was found in several experiments that indicated a current spread of less than 1 mm. Some of the locus coeruleus stimulation sites are shown separately in other parts of the present investigation from individual experiments.

The effect of locus coeruleus stimulation (P2-P4) produced similar effects on the discharge of the multirecpetive neurones.

Location of NRM stimulation sites
(Parts A-D)

The NRM stimulation sites producing various effects on the discharge of multireceptive neurone are shown as filled triangles lying within or adjacent to the nucleus raphe magnus (Fig. 4.12, P6-P8).

The nucleus was stimulated at various sites along its rostrocaudal extent from P6-P9. At each rostrocaudal co-ordinate all the sites of stimulation (Fig. 4.12) that produced various effects already described are shown for the present investigation (Parts A-D). From the entire rostrocaudal extent of the nucleus investigated similar effects were obtained. The sites outside the nucleus required higher stimulus intensities to produce the same effects than the sites which were located within the nucleus. However it would not be possible to speculate about the spread of current laterally to the structure lying lateral to the nucleus since that area (nuclei reticularis magnocellularis and gigantocellularis) also projects to the spinal cord. The nucleus reticularis magnocellularis (RMc) which is situated ventromedially in the medulla lying laterally to the NRM and has a similar projection pattern to the NRM projections. However the projections from NRM are bilateral to the spinal cord whereas the projections from RMc are ipsilateral. As will be pointed out in Part C, to abolish NRM effects requires a bilateral DLF lesion indicating that the effects from NRM stimulation were not due to the current spread to the surrounding structures. The NRM effects could be evoked at current intensities less than 50 μA from within the centre of the nucleus, placed even 2 mm dorsal to the pyramidal tract. This finding also ruled out the possibility of current spread to the pyramidal tract.

PART B

Involvement of putative transmitters in mediating descending effects from LC and NRM

The various actions of locus coeruleus and raphe magnus stimulation have already been covered in Section A where the occurrence of predominantly inhibitory actions from LC and NRM have been described. In order to study the involvement of various putative transmitter substances in mediating these descending effects, pharmacological studies were conducted. The nucleus locus coeruleus, until recently, was thought to be only a catecholamine containing nucleus but recent investigations have shown the presence of other substances such as substance-P and neurotensin in neurones of the nucleus locus coeruleus. Similarly the concept of the midline raphe complex containing only 5-HT has also been invalidated by the demonstration of substance-P and Met-enkephalin in this region which may co-exist in the 5-HT containing neurones. This part of the investigation was carried out in keeping with this existing knowledge to study the involvement of catecholaminergic, serotonergic, Gabaergic and opioid mechanisms in mediating descending actions on multireceptive neurones exerted from LC and NRM. The involvement of catecholamines in mediating LC actions was studied by administering intravenously the antagonists for the α-receptor (Phenoxybenzamine, Yohimbine), and the β-receptor (Sotalol, Propranolol) on the premise that if catecholamines were involved then an antagonism of LC effects on the discharge of multireceptive neurones will be observed. The serotonin antagonists, methysergide and cinnanserin, were administered not only to study the involvement of serotonin in mediating NRM actions but also to find an answer to the question of pathways involved in mediating these actions. The specific opioid antagonist, naloxone, and the GABA antagonist, bicuculline, were injected intravenously for studying the involvement of opioid and Gabaergic mechanisms as well as the possible differentiation of the

chemical nature of these descending pathways that might be revealed which could provide some clues to the pathway or pathways from LC and NRM.

Antagonism by phenoxybenzamine and yohimbine of the LC inhibition produced on the discharge of multireceptive neurones

The effect of phenoxybenzamine (PBZ) (2 mgm/kg i.v.) was tested on the LC inhibition of the discharge of three multireceptive neurones in three cats. The antagonism of LC inhibition was observed on two of these neurones whereas the remaining one neurone was lost during PBZ administration. The antagonism of LC inhibition by PBZ is shown in Fig. 4.13. The stimulation in the nucleus locus coeruleus inhibited the evoked discharge to noxious heat (46°C) by 70% as well as the background discharge by 75-80%. The antagonism of the LC inhibition produced by phenoxybenzamine started 10 minutes after its administration. This reduction in the LC inhibition produced by phenoxybenzamine continued to rise and reached a peak value within 50 minutes (Fig. 13). However the inhibitory effect of LC on the spontaneous discharge was not changed by phenoxybenzamine. Although 20 minutes after the administration of the drug it looked as if there was some antagonism (Fig. 4.13), comparing it with the percentage variability of the control response (Fig. 4.13) did not reveal any significant difference. This particular unit was observed over a period of longer than three hours but still did not show any significant reduction in the LC-evoked inhibition of the background discharge. However an almost complete reversal of the LC inhibition of the evoked response to heat was observed (Fig. 4.13). A similar pattern was observed in the second neurone as well.

Phenoxybenzamine was administered slowly over a period of 10-15 minutes in order to minimize the sudden hypotensive effect of the drug. Although as shown in Fig. 4.21 D the systolic blood pressure dropped from 150 to 100 mm Hg, yet it did not cause any change in the shape or size of the spike and there was also no change in the spontaneous or the evoked discharge to heat.

Antagonism of LC inhibition by yohimbine

Yohimbine was injected intravenously (1-2 mgm/kg) over a period of 5-10 minutes to minimize the sudden drop in the blood pressure (Fig. 4.21 C). The effect of yohimbine was tested on the LC evoked inhibition on three neurones in three animals. The inhibition was significantly reduced on two, whereas the remaining one unit was lost shortly after the intravenous injection of yohimbine. Before the drug was administered, LC stimulation produced a complete suppression of the evoked response to pinch (Fig. 4.14) whereas after the yohimbine administration the inhibition started decreasing within 10 minutes and a significant reduction in the inhibition was observed at 25 minutes. It looked as if the LC evoked inhibition started reappearing at 35-40 minutes but nothing categorical can be said since the units were lost after that period. The threshold for producing inhibition from LC was 100 μA. The inhibition of the discharge of the multireceptive neurone produced by the stimulation in LC at the threshold intensity was completely reversed by yohimbine. The change in the control response evoked by noxious pinch after yohimbine was possibly due to the repeated application of the noxious stimulus (Fig. 4.14).

Effect of sotalol and propranolol on the LC produced inhibition on the discharge of multireceptive neurones

The effect of β-noradrenergic blockers sotalol and propranolol (1-6 mgm/kg i.v.) was tested on the LC produced inhibition on four multireceptive neurones in four cats. There was no reduction in the inhibition produced by stimulation of LC either on the discharge evoked by noxious stimuli or on the spontaneous activity. However propranolol (1 mgm/kg) did cause (2 neurones) a marked increase in the response evoked by low threshold cutaneous stimulation and also increased the response produced by pinch (Fig. 4.15 a, b) but there was no change in the inhibition produced from LC (Fig. 4.15 a, b). Similarly sotalol (1-6 mgm/kg) did not produce any antagonism of the inhibitory effect produced by the stimulation in the locus coeruleus (Fig. 4.15 C). It produced a transient hypotensive effect which returned to normal level within six minutes (Fig. 4.21 B).

Effect of naloxone on the inhibition produced by the stimulation of LC and NRM on the activity of multireceptive neurones

Naloxone (0.2-3 mgm/kg) was tested on the inhibitory effect produced from stimulation in LC and NRM on the discharge of 5 multireceptive neurones in five cats. One of these units was located superficially in lamina I whereas the remaining units were located in the deeper laminae V and VI. The inhibition of four of these multireceptive neurones produced by the stimulation in LC was partially reduced by naloxone. The inhibition evoked by the stimulation of NRM was however partially reduced only on one neurone after naloxone administration. However, on another unit (Fig. 4.18 a),

naloxone did decrease the early part of the inhibition and enhanced the later period of the inhibition produced by the stimulation of NRM (Fig. 4.18 b). Naloxone produced an increase in the spontaneous activity, evoked discharge to heat or pinch and also increased the number of spikes in the C-fibre discharge of a multireceptive neurone. In this particular unit stimulation of NRM produced a reduction of 60% in the early A-fibre evoked discharge whereas a 90% reduction in the later C-fibre evoked discharge was observed (Fig. 4.18 a). The stimulation of locus coeruleus produced a reduction of 80% in the early (A-fibre) discharge and a 90% reduction in the later C-fibre evoked discharge (Fig. 4.18 a). After naloxone administration (1 mgm/kg) there was a partial reduction of 30% in the inhibition of both the A and C-fibre evoked discharge produced by stimulation in the nuclei locus coeruleus and raphe magnus (Fig. 4.18 b).

On another neurone located in the superficial part of the dorsal horn (lamina I) an early period of complete inhibition (200 m sec) (Fig. 4.19 a) produced by LC stimulation was reduced to 120 m sec after naloxone administration (1 mgm/kg) (Fig. 4.19 b). On the same neurone however different effects were observed after naloxone injection on the inhibition produced by the stimulation of NRM. The earlier 200 m sec period of complete inhibition produced by NRM stimulation (Fig. 4.19 a) was reduced but the subsequent period (600 m sec) showed a greater inhibition (Fig. 4.19 b) after naloxone administration. The effects produced by naloxone on the inhibition from LC and NRM were observed within three minutes and a complete recovery was observed within 70 minutes. Three different periods of inhibition were observed on another multireceptive neurone produced by the electrical stimulation of LC and NRM. The nucleus

raphe magnus produced two periods of inhibition at 120 and 400 m sec. whereas LC stimulation in addition produced another period of inhibition from 1000-1500 m sec. The middle part of the inhibition from LC was abolished by naloxone but it did not produce any effect on the NRM inhibition. A partial reduction in the inhibition from LC produced on the background discharge of a multireceptive neurone was observed on one neurone whereas it did not change the inhibition from NRM.

Effect of methysergide and cinnanserin on the actions produced by the electrical stimulation of LC and NRM on the discharge of multireceptive neurones

Methysergide (0.5-15 mgm/kg) did not produce any effect on the inhibition produced from LC or NRM on the activity of six neurones tested in six cats. It did not antagonise the inhibition produced from LC or NRM on the spontaneous or the evoked discharge of any of the six multireceptive neurones tested (Fig. 4.16, 4.17). However methysergide did antagonise the excitation produced by NRM stimulation on a multireceptive neurone (Fig. 4.17 a, b). The drug administration also caused changes in the spontaneous activity of the cell (Fig. 4.17 b), it almost completely abolished the background discharge and also produced a small increase in the blood pressure (Fig. 4.21 b) which returned to normal level within 10-15 minutes.

Another 5-HT antagonist cinnanserin (1-4 mgm/kg) was also tested on the inhibition produced by stimulation in the LC and NRM on the activity of two multireceptive neurones in two cats. This

antagonist also failed to antagonise the inhibition produced by the stimulation of LC and NRM on the discharge of multireceptive neurones.

Bicuculline antagonism of LC and NRM actions produced on the discharge of multireceptive neurones

The effect of bicuculline (0.5 mgm/kg) was tested on the LC and NRM actions exerted on the discharge of three multireceptive neurones in three cats. There was an immediate rise in the blood pressure after bicuculline which returned to normal level within 5-10 minutes (Fig. 4.20 E). The bicuculline antagonism of the inhibitory effect of LC and NRM was observed on all the three multireceptive neurones tested. One of these multireceptive unit showed wind-up to inhibition produced by the stimulation of LC and NRM (Fig. 4.20 a) but did not show any wind-up in the early excitation. Bicuculline administered over a period of one minute not only abolished the DRPs but also the inhibition from LC and NRM (Fig. 4.20 b). The inhibition from LC and NRM started reappearing 17 minutes after the administration of bicuculline. The correlation between the abolition and reappearance of the LC and NRM evoked DRPs as well as the antagonism of the inhibition produced by stimulation in LC and NRM on multireceptive neurones by bicuculline administration will be described under Section D. The inhibition evoked from LC and NRM on the discharge of multireceptive units was abolished within 2 minutes of bicuculline injection and started reappearing within 15 to 30 minutes. There was only one unit which was recorded on the border between laminae II and III which was excited by low threshold mechanosensory input and was inhibited by the high threshold mechanical

noxious stimulus applied to the same place. The inhibitory and excitatory fields overlapped. The inhibition evoked on this unit from LC and NRM was also antagonised by bicuculline. The spontaneous activity of this unit was also abolished by bicuculline. In the remaining two units opposite effects were observed on the background activity of the multireceptive units after bicuculline. The discharge of one of the units increased whereas it was reduced on the other unit after bicuculline.

PART C

Contribution of direct as well as indirect pathways through other brainstem nuclei such as the nucleus raphe magnus in mediating the locus coeruleus modulation of the spinal sensory transmission

Comparison of the effects of LC and NRM stimulation on the activity of the multireceptive neurones

It has already been described in the results under Section A that the effect of LC and NRM stimulation was tested and compared on the activity of the same multireceptive neurones. The effects of LC and NRM stimulation were tested and compared on the spontaneous and evoked activity of 92 multireceptive neurones. These results as already mentioned (Section A) revealed qualitatively and in most cases quantitatively similar actions from LC and NRM. That part of the investigation thus demonstrated that the activity of a single multireceptive neurone could be modulated from both LC and NRM. The question then arose if the LC evoked actions are mediated through the nucleus raphe magnus, through some common unknown pathway from both or through the direct coeruleospinal cprojections. The funicular trajectories of descending fibres to the spinal cord are mostly different from both LC and NRM. In order to investigate these various questions an attempt was made to make selective lesions in the upper lumbar and the lower thoracic region of the spinal cord which might differentiate the pathways mediating these effects from LC and NRM. Electrolytic lesions in the nucleus raphe magnus which extended to the caudal raphe nuclei as well were also made on the premise that if NRM is the mediator of LC actions then abolition of its actions would be observed after the midline raphe lesions.

Experiments reported in this section are from 8 cats. In four of these experiments recordings were made from 51 multireceptive neurones and the effect of making various lesions mainly in the lower

thoracic region of the spinal cord was observed on the actions produced by the stimulation of LC and NRM on the activity of multireceptive neurones. The nucleus raphe magnus was lesioned in two cats and the effect of LC stimulation was studied on the activity of 14 multireceptive neurones before and after the lesions. In the remaining two cats an attempt was made to isolate the pathways by making lesions in the lower thoracic and upper lumbar spinal cord and observing the effect on the DRPs evoked from LC and NRM.

Effect of an ipsilateral hemisection on the descending effects produced from LC and NRM

Before making an ipsilateral hemisection in the lower thoracic spinal cord the effects of LC and NRM stimulation were tested on the spontaneous and evoked activity of several neurones (10) in the lumbar spinal cord. Predominantly inhibitory effects were observed before the lesion but mixed early excitation followed by a period of complete inhibition was also observed (Fig. 4.22 A). The periods of inhibition lasting as long as 1 second were observed from LC and NRM on neurones before the lesion was made. After an ipsilateral hemisection was made (shown by the black area on the right-hand side of the Fig. 4.22 B, C) the inhibitory effect produced by LC stimulation was abolished (Fig. 4.22 B). However on the same neurone after the lesion was made there was still an initial period of complete inhibition lasting for <200 m sec produced by the stimulation of nucleus raphe magnus (NRM). An ipsilateral cord hemisection produced an abolition of LC produced inhibition whereas the NRM inhibition was reduced to a considerable extent. This indicated the

ipsilateral location of the pathway mediating the actions produced by the stimulation in LC whereas a bilaterally located pathway was indicated in mediating the effects from NRM.

Effect of selectively lesioning various parts of the lower thoracic spinal cord on the modulation of the spinal cord activity produced by the stimulation of LC and NRM

The effect of the locus coeruleus and nucleus raphe magnus stimulation before making any lesions was tested on the discharge evoked by the peripheral electrical (Fig. 4.23 A) and noxious cutaneous stimulation (Fig. 4.23 B) as well as on the background discharge (Fig. 4.23 C). The effect of LC and NRM stimulation was tested on the discharge of six multireceptive neurones before any lower thoracic lesions were made. The stimulation of LC and NRM before the lesions produced an inhibition, or mixed excitation and inhibition on multireceptive neurones (Fig. 4.23 A, B). The inhibitory, or the mixed excitatory and inhibitory effects were observed from LC and NRM on all the six neurones tested before the lesions were made in the same animal. After making the first thoracic lesion in this experiment (Fig. 4.24 A, B, right-hand side) the stimulation of LC did not produce any visible inhibition but still produced the excitation (Fig. 4.24 A). The stimulation of NRM still produced excitation and inhibition of the discharge of this neurone evoked by electrical stimulation (Fig. 4.24 A). However on testing the effects from LC and NRM on the activity of another unit evoked by the noxious pinch revealed the mixed early excitation and an inhibitory effect from both LC and NRM (Fig. 4.24 B). The lesion involved the complete

ventral funiculus and also a part of the ventrolateral funiculus (VLF) (Fig. 4.24).

After making the first lesion (Fig. 4.24), a dorsal horn multireceptive neurone was held in the same experiment which was held throughout the lesions which were made subsequent to the lesion already mentioned. These various lesions are shown against each record which was obstained following each lesion (Fig. 4.25 a, b, c). The black areas in the spinal cord transverse sections show the extent and the lesion made before the record was taken and the hatched areas represent the lesions made previous to the present lesion (black area). The response of this neurone to tibial nerve stimulation (20 Volts) is shown (Fig. 4.25 a) after making the lesion shown on the right-hand side. This multireceptive receptive neurone showed wind-up to tibial nerve stimulation and the wind-up was particularly marked for the C-fibre evoked discharge (Fig. 4.25 a). After an almost complete lesion of the ipsilateral VLF and VF no inhibition, or mixed excitation and inhibition was observed from LC (Fig. 4.25 a) but the inhibitory effect produced by the stimulation of NRM was still pronounced, particularly, on the C-fibre evoked discharge (Fig. 4.25 a). On making the next lesion which involved the ipsilateral dorsolateral funiculus (DLF) (Fig. 4.25 b) reduced the inhibitory effect from NRM on the C-fibre evoked discharge of this neurone (Fig. 4.25 b). After having made the above lesions (Fig. 4.24; 4.25, a, b) another lesion was made subsequently in this animal which sectioned the contralateral dorsolateral funiculus (DLF) (Fig. 4.25 c). The remaining inhibitory effect produced from NRM on the C-fibre evoked discharge was also abolished (Fig. 4.25 c). The

stimulation of LC did not produce inhibition of the A or the C-fibre evoked discharge after the second lesion (Fig. 4.25 a). The earlier part of the response evoked by various components of the A-fibre was not very consistent in the controls (Fig. 4.25 a, b, c). Similarly though NRM still exerted an inhibitory effect on the C-evoked evoked discharge (Fig. 4.25 b) yet there did not seem to be any effect on the A-fibre evoked discharge. The recording site of this unit is shown in Fig. 4.26 a.

These particular results from a single experiment demonstrate that the pathway mediating the LC effects was located in the ipsilateral ventral quadrant (VLF + VF) whereas the pathway mediating the NRM actions was located in the DLF bilaterally. The recording and stimulation sites in LC and NRM of this experiment are shown in Figure 4.26 (a, b, c).

In one of the four experiments 13 neurones were recorded prior to placing any lesion in the spinal cord. The effects of LC and NRM stimulation ranged from inhibition, or mixed excitation and inhibition on the multireceptive neurones. After an ipsilateral hemisection, stimulation of the locus coeruleus still produced an excitation but did not produce inhibition whereas stimulation of NRM produced inhibition but no excitation on one of the neurones. On one of the remaining two neurones tested after this lesion, LC stimulation did not produce any action whereas NRM stimulation caused an inhibition lasting longer than 1 second. The discharge evoked by pinch of another multireceptive neurone was inhibited from LC for a period

of 150 m sec whereas NRM stimulation inhibited it for a period of >300 m sec. On making a subsequent contralateral DLF lesion the NRM inhibition was reduced considerably whereas the LC inhibition though considerably diminished yet lasted for a period of less than 50 m sec.

Effect of lesioning the dorsal half of the spinal cord in the lower thoracic region on the inhibition produced by the stimulation of LC and NRM on the discharge of a multireceptive neurone held throughout the lesion

One of the multireceptive neurones was held throughout the period of testing, before and after making a lesion which involved the DLF bilaterally and the dorsal columns (Fig. 4.27). The stimulation of LC and NRM, prior to the lesion, inhibited the spontaneous discharge of this cell (Fig. 4.27, top part) whereas after the lesion the LC produced inhibition was still the same but only a small percentage of the inhibition was left from NRM (Fig. 4.27, bottom part). In this particular experiment, prior to the lesion, all the 10 multireceptive neurones tested for effects produced by the stimulation of LC and NRM showed inhibition from both the nuclei whereas after the lesion two neurones tested did show a similar magnitude of inhibition from LC but the inhibition from NRM was reduced significantly on one and was completely abolished on the other.

Effect of lesioning the midline raphe nuclei including NRM on the actions produced by LC stimulation on multireceptive neurones

In two cats lesions involving almost the whole of NRM and the caudal raphe region were made to investigate if these lesions will abolish or reduce the actions exerted on multireceptive neurones from LC or not. This will provide additional information if the LC actions on the multireceptive neurones in the spinal cord are mediated through a relay in the NRM or not. On seven multireceptive neurones the stimulation in LC (Fig. 4.28 D) and NRM produced an inhibition of the spontaneous as well as the evoked discharge prior to the lesion (Fig. 4.28 A). The extensive midline lesions represented by the stippled area within the solid lines (Fig. 4.29) were made from P5-P13. These lesions covered almost the entire area which includes the NRM. The broken lines represent an area of the midline which might have escaped the lesions. Only at the most caudal parts (Fig. 4.29, P11-P12) some parts of the midline region were not lesioned completely. After making the extensive midline raphe lesions the effect of LC stimulation was tested on the spontaneous (Fig. 4.28 B) and the evoked response to heat (Fig. 4.28 C) and produced a similar magnitude of inhibition in all the three neurones tested after the lesions. After the lesions were made, the NRM electrode was placed at various points from P5-P9 to study if any actions were exerted by stimulating NRM at these sites which provided a useful indication for the accuracy of the lesions made. On no occasion was there any effect exerted from any of these places in the NRM on the multireceptive neurones (Fig. 4.28 C) after the midline lesion which was expected on the assumption that lesions were complete.

In the other experiment the lesions were not as complete as shown for the experiment already described (Fig. 4.29) and slightly laterally placed lesions at some places along the rostrocaudal co-ordinates P5-P13 were found later in the transverse sections. Two units tested before the lesion showed inhibitory effects from LC and NRM and one of these units was held during the period the lesions were being placed in the midline raphe region and for some time after that. After the midline lesions the unit was inhibited by LC stimulation but higher repetition rate of a train of stimuli from 2 to 5 HZ had to be used. The spontaneous discharge of this unit increased considerably after the lesions. All the natural cutaneous stimuli, pinch, noxious heat as well as the low threshold stimulation applied to the same parts of the receptive that elicited excitation prior to the lesions, evoked inhibition of the spontaneous discharge after the lesions. The effect of electrical stimulation of the dorsal root also changed from excitatory to inhibitory after the midline lesions. However the stimulation in LC still produced an inhibition of the background discharge.

These two experiments indicate that the NRM is not involved in mediating the actions produced from LC on the multireceptive neurones. It also indicates that a region situated lateral to the midline raphe complex together with the midline raphe region might be a potent site exerting tonic descending control on the spinal cord neurones.

Effect of lesions placed in the lower thoracic region of the spinal cord on the DRPs evoked from LC and NRM

Since the stimuli evoking inhibition from LC and NRM also produced DRPs the effect of placing lesions in the lower thoracic and upper lumbar spinal cord was tested on the DRPs recorded from a rootlet from the L_7 dorsal root. In two such experiments the DRPs were studied before the lesions and were then compared with the DRPs after making lesions. In one experiment the lesion made in the ipsilateral DLF (Fig. 4.30 a) did not produce any change in the DRP from LC or NRM. However when a subsequent contralateral hemisection (black area) was made (Fig. 4.30 b) the DRP from NRM was abolished whereas the DRP evoked by LC stimulation was still present (Fig. 4.30 c). The third lesion made subsequently represented by the black area (Fig. 4.30 d) abolished the LC evoked DRP. The hatched areas in each spinal cord section represent the lesions made prior to the present lesion represented by the black area. It showed that the NRM evoked DRP was generated through a pathway in the ipsilateral DLF and the contralateral side (Fig. 4.30 a, b, c) whereas the LC evoked DRP was produced through a pathway possibly located in the ipsilateral VLF (Fig. 4.30 d). The recording system picked up the 50 cycles which is visible in the records of DRPs (Fig. 4.30 c).

In another experiment a more detailed analysis of the effect of lesions on the DRPs evoked from LC and NRM was carried out. The thresholds for DRPs from LC and NRM were respectively 85 and 65 μA (Fig. 4.31 a). After making the first lesion, shown in black on the right-hand side of Fig. 4.31 b which involved the dorsal half of the

spinal cord in the thoracic region the DRPs evoked from LC and NRM at a higher stimulus intensity (300 μA) are shown after the first lesion (Fig. 4.31 b). After the first lesion, on making a subsequent lesion that involved a part of the ipsilateral VLF and VF (Fig. 4.31 c) reduced the DRPs produced from both LC and NRM (Fig. 4.31 c) but decreased the LC evoked DRP to a greater extent. The next lesion (Fig. 4.31 d) which involved the ipsilateral ventrolateral and ventral funiculus (VLF and VF) abolished the DRP from LC but the stimulation of NRM still produced a DRP similar to the one shown in Fig. 4.31 c. The next lesion (Fig. 4.31 c) which involved contralateral side of the cord abolished the NRM evoked DRP. The hatched areas in the spinal cord sections placed on the right-hand side of each figure represent the lesions made previous to the present lesion shown in black.

The results indicate that the DRPs evoked from LC and NRM are generated through separate pathways. The descending pathway generating DRP from LC is located in the ipsilateral ventral quadrant (VLF and VF) whereas the NRM evoked DRP is produced by a pathway located bilaterally in the spinal cord.

PART D

Contribution of pre- and postsynaptic mechanisms underlying the modulation of the spinal sensory transmission from the nucleus locus coeruleus

It has already been pointed out in results described under Section A that the LC stimulation produced both a selective and non-selective reduction in the discharge of multireceptive neurones evoked by natural cutaneous stimulation. In fifty percent of the sample tested (5 of 10) a selective inhibitory action on the discharge evoked by the nociceptive input of these neurones was observed, leaving the non-noxious cutaneous input unchanged. However in the remaining units (5) a non-selective reduction in the discharge produced by both the noxious and non-noxious cutaneous inputs was observed (Fig. 4.7 a). Although even on observing non-selective inhibitory action, the inhibitory effect of LC was more pronounced on the nociceptive input as compared to the non-nociceptive input on three neurones (Fig. 4.7 a). In the remaining two units the inhibition from LC was of the similar magnitude on the discharge evoked by both the noxious and non-noxious cutaneous stimulation. The LC stimulation also reduced the early A-fibre and late C-fibre evoked discharge of several neurones. Mixed effects, an early excitation followed by a prolonged period of inhibition were also observed from LC and NRM.

These results indicate the possible involvement of pre-synaptic and postsynaptic mechanisms. In order to resolve the question of the involvement of postsynaptic inhibitory mechanisms in producing LC actions intracellular recordings were made from multireceptive neurones after the completion of extracellular analysis of the LC inhibition on a neurone. Several multireceptive neurones were impaled intracellularly but so far only two stable intracellular penetrations allowed the proper analysis to be carried out. A stable

40-50 mV DC shift was observed on advancing the electrode intracellular from the extracellular recording position. The intracellular recording from a multireceptive neurone (Fig. 4.32 b) showed a long lasting (>300 m sec) IPSP having an amplitude of <10 mV produced by the stimulation of the nucleus locus coeruleus. The extracellular recordings carried out prior to the intracellular recording in this cell, revealed a period of inhibition lasting for 200-300 m sec. This particular neurone was also inhibited from another brain-stem nucleus, reticularis magnocellularis (RMc) which is located lateral to the nucleus raphe magnus in the ventromedial medulla. The period of inhibition produced from this nucleus was 200 m sec and the intracellular recording revealed that the IPSP lasted for up to 100 m sec (Fig. 4.32 c). The IPSP was not very clear from this nucleus at a lower gain but at a higher gain it became more clear (Fig. 4.32 d). On superimposing five traces the IPSP still appeared at the same place (Fig. 4.32 d). Even on superimposing 10 intracellular traces the IPSP appeared at the same place. A small hyperpolarisation visible on the spikes could reflect the after hyperpolarisation or some sort of sub-synaptic potentials. EPSPs evoked by the dorsal root stimulation were also seen (Fig. 4.32 a) but so far the effect of LC and NRM stimulation has not been tested on the EPSPs or the IPSPs evoked from the peripheral stimulation. The central stimulation (LC) evoked EPSPs have also been recorded. The stimulation sites that evoked IPSP from LC and RMc (Fig. 4.32 b, c, d) are shown in Figure 4.32 e, f.

Occasionally on intracellular penetrations no IPSPs were recorded by stimulation of LC or NRM. However nothing was known about the cutaneous input of these cells or if LC, NRM or RMc exerted any effects on these cells or not since no extracellular recordings were made prior to the impalement of the cell. The withdrawal of the electrode from the intracellular to the extracellular position however killed the cells. IPSPs evoked from the peripheral electrical stimulation have also been recorded from some neurones.

Pre-synaptic inhibition

A small part of the dorsal root was placed on a bipolar Ag-AgCl electrode for recording the evoked dorsal root potentials (DRPs) from LC and NRM. The stimulation of LC and NRM evoked DRPs in all the 7 experiments conducted on DRPs. The thresholds for DRPs from LC and NRM were sometimes found to be similar (Fig. 4.31 a) whereas it was different on other occasions. The current intensities that produced inhibition from LC and NRM also evoked DRPs.

Effect of bicuculline on the DRPs as well as the inhibition evoked from LC and NRM

The DRPs evoked from LC and NRM (Fig. 4.33 b) were abolished (Fig. 4.33 c) by bicuculline (0.5 mgm/kg) within 1 minute of the intravenous administration in all the four experiments in which it has been tested so far. It also antagonised the inhibitory effect produced by stimulation of LC and NRM on three neurones tested in three of the experiments (Fig. 4.20 b). In these experiments

bicuculline also abolished the DRPs evoked from LC and NRM. After abolition by bicuculline the DRPs start reappearing within 15-30 minutes which correlated with the reappearance of the inhibition on the cell from the brain-stem. A complete recovery of DRPs after bicuculline was observed within 70 minutes (Fig. 4.33 d). These results indicate that not only was there an antagonism of evoked DRPs generated from LC and NRM but also there was an antagonism of the inhibition of multireceptive neurones produced from LC and NRM by bicuculline administration. The time course of onset of this antagonism of the inhibition as well as the time course of the reappearance of the inhibition from LC and NRM after bicuculline was similar. This indicates the possible involvement of GABA in the generation of both the DRPs and the inhibition from LC and NRM.

DISCUSSION

The electrical stimulation of various brain structures including the PAG and the medial brain-stem have been shown to produce behavioural analgesia and considerable evidence has been provided to show the existence of endogenous pain suppression systems (Mayer and Price, 1976; Basbaum and Fields, 1978; Fields and Basbaum, 1978; Yaksh and Rudy, 1978). The sites producing analgesia by the microinjection of morphine or by the electrical stimulation overlap to a considerable extent, both producing a potent analgesia from the ventrolateral periacqueductal gray matter (PAG). The nucleus locus coeruleus, lying in the dorsolateral tegmentum in the pons and classically known as a catecholamine containing nucleus has been reported to produce analgesia on electrical stimulation (Segal and Sandberg, 1977; Sandberg and Segal, 1978) as well as by the microinjection of morphine (Yaksh and Rudy, 1978). The mechanisms underlying the analgesia produced from LC and the dorsal raphe are unknown. The physiological significance of the descending projections to the spinal cord from the nucleus locus coeruleus (Kuypers and Maiskey, 1975, 1977; Hancock and Fougerousse, 1976; Nygren and Olson, 1977; Tohyama et al. 1979; Westlund and Coulter, 1980) together with the mechanisms involved in producing analgesic actions from LC are some of the features which should be discussed based on the evidence provided by the present investigation. The discussion will revolve around the results obtained together with the speculative possibilities underlying the various mechanisms that might contribute to the descending control of the sensory transmission, in particular, the nociceptive transmission from the nucleus locus coeruleus (LC). This

will help not only to unmask the physiological significance of this pathway in the spinal cord but also on the mechanisms underlying the analgesia produced from LC.

The findings described in the present investigation make one fact outstandingly clear, that the electrical stimulation in the nucleus locus coeruleus produces a predominantly inhibitory effect on the discharge of the spinal cord multireceptive neurones. On some neurones, however, mixed effect of excitation and inhibition, or excitation alone have also been observed. A selective inhibitory action on the noxious input as well as a non-selective reduction in the discharge of multireceptive neurones have also been observed. The nucleus coeruleus however produced only an inhibition of the activity of specific nociceptor driven neurones. The majority of the low threshold mechanoreceptive neurones was not affected but on a few neurones however either an inhibitory or an excitatory action was produced by the stimulation of LC. The results reported in the present investigation are not different from the findings of a recent brief report that described the predominance of inhibitory effect of LC stimulation on the discharge of the spinal cord neurones (Hodge et al. 1981). A large proportion of the sample of mechanoreceptive neurones were reported to be inhibited (Hodge et al. 1981) whereas only a few such neurones were observed to be inhibited in the present investigation. The discrepancy could very well lie in the small sample (10 neurones) of the low threshold mechanoreceptive neurones examined in the present study. The noradrenergic-mediated inhibitory effect of the nucleus locus coeruleus stimulation have been observed on neurones in the spinal trigeminal nucleus in the cat (Sasa and

Takaori, 1973; Sasa et al. 1974) and a feedback loop between the locus coeruleus and spinal trigeminal nucleus neurones has been suggested in the rat (Igarashi et al. 1978). It is interesting in this regard that a similar inhibitory effect which predominated the excitatory action has also been observed on stimulation of locus coeruleus on the spontaneous discharge of neurones in the visual cortex in the rat (Olpe et al. 1980) and these effects were shown to be mediated by the β-noradrenergic receptor. However locus coeruleus stimulation produced facilitation of the lumbar monosynaptic reflexes has been shown to be mediated by the α-adrenergic receptor in the cat (Strahlendorf et al. 1980). It has also been shown recently that the inhibition of the extensor monosynaptic reflex produced by stimulation of high threshold muscle or cutaneous afferents was inhibited by the rhythmic stimulation in the locus coeruleus (Yakhnitsa and Pilyavsky, 1980 a) whereas the facilitation of the lumbar flexor monosynaptic reflex produced by volleys in high threshold cutaneous and muscle afferents was not changed by stimulation in the nucleus locus coeruleus. The stimulation of locus coeruleus can not only exert a selective inhibitory action on the discharge evoked by the noxious stimulation of a multireceptive neurone observed in the present investigation but it can also exert a selective modulatory influence on the reflex transmission by suppressing the inhibitory action produced by volleys in the high threshold cutaneous afferents, leaving the excitatory action unchanged on the extensor monosynaptic reflex (Yakhnitsa and Pilavsky, 1980 a).

The findings of the present study as well as the evidence reported by other investigators demonstrate that the nucleus locus coeruleus can not only exert an inhibitory control on the spinal cord nociceptive transmission but also on the inhibitory action evoked by the high threshold cutaneous afferents on the extensor monosynaptic reflex (Yakhnitsa and Pilavsky, 1980 a, b). The spinal cord nociceptive reflexes have been reported to be inhibited by the stimulation produced analgesia (Mayer et al. 1971; Mayer and Liebeskind, 1974; Mayer and Price, 1976). Although the inhibition of the spinal cord nociceptive reflexes does not necessarily mean that the spinal cord nociceptive transmission is blocked yet it does provide an indication that some common neural mechanisms underlie the nociceptive reflexes, nociceptive transmission and the perception of pain. The findings of Matsushita (1969) show axons of some dorsal horn neurones in lamina V that enter the lateral cord and some of their collaterals terminate in the ventral horn. This provides support for such a suggestion as that of a common neural mechanism underlying the spinal nociceptive transmission and the spinal cord nociceptive reflexes.

Some of the spinal cord multireceptive neurones have been shown to project to the thalamic nuclei (Section II) and the spinothalamic tract in the primate has been demonstrated to contain a large proportion of these neurones in its composition (Willis, 1981). On the assumption that the cells of the spinothalamic tract have similar anatomical characteristics as described by Matsushita (1969), inhibition of the monosynaptic extensor reflex evoked by volleys in the high threshold cutaneous afferents can be produced if an inhibitory

interneurone in the pathway from the collateral of a projecting neurone to the motoneurone is imagined. In such a speculative situation as that, locus coeruleus stimulation-produced inhibition of a projecting multireceptive neurone or an interneurone in its pathway can also account for the inhibition of the inhibition produced on the extensor monosynaptic reflex by volleys in the high threshold cutaneous afferents. Similarly the inhibitory effect produced by stimulation in the locus coeruleus can also be exerted on the common internuncial pools shared by the spinal cord nociceptive reflexes and the spinal cord neurones transmitting nociceptive transmission to the thalamic nuclei or the reticular formation. The spinal cord multireceptive neurones have been shown to project to thalamic nuclei in the cat (Section II) and the primate spinothalamic pathway contains a substantial proportion of these neurones (Price et al. 1978; Willis, 1981). The first and second pain is evoked in humans by single electrical or heat pulse and this has also been shown to produce a short and long latency response in the multireceptive spinothalamic tract neurones in the primate (Price et al. 1978). The investigators also reported that a tactile stimulation produced a prolonged discharge in the spinothalamic neurones and the same stimulus applied to the human foot produced after-sensation. Price and Mayer (1975) from their combined electrophysiological and behavioural studies have suggested that the multireceptive neurones play an important part in pain transmission. In view of this evidence it is reasonable to suggest that one of the neural mechanisms underlying the analgesia produced from the locus coeruleus is by producing a suppression of the nociceptive transmission as has been revealed in the present investigation. The stimulation produced

analgesia from LC could also be mediated in part by the inhibitory action it can exert at various levels of the neuraxis which are involved in the processing of nociception transmission and the perception of pain. It has been reported that the stimulation of the nucleus locus coeruleus causes a predominantly prolonged inhibitory effects in the cerebellum, hippocampus, diencephalon and cortex (Moore and Bloom, 1979) which are mediated by the β-noradrenergic receptor, possibly coupled to an adenylate cyclase.

The observation of the LC evoked inhibition of the spinal cord specific nociceptor driven neurones (lamina I) in the present investigation will also be important in mediating analgesia reported to be produced by stimulation in LC. It is difficult to understand the significance of the inhibition or excitation of some low threshold mechanoreceptive neurones in contributing to analgesia produced from LC but enhanced activity along non-nociceptive pathways can be envisaged to gate the nociceptive transmission at several levels of the neuraxis. However the non-selective inhibitory effect exerted on multireceptive neurones together with the inhibition of the non-nociceptive cells may also indicate the significance of these results for the other physiological functions such as sleep which will be discussed towards the end. The detailed mechanisms underlying the various actions evoked from LC will be discussed in the later part of the discussion.

The stimulation of the nucleus raphe magnus (NRM) has been shown to produce analgesia and it has been suggested that NRM is the common mediator of the stimulation produced analgesia (SPA) as well as the morphine produced analgesia (Mayer and Price, 1976; Liebeskind

and Paul, 1977; Fields and Basbaum, 1978; Basbaum and Fields, 1978; Fields and Anderson, 1978). The nucleus raphe magnus has been shown to project bilaterally through the DLF to the spinal cord (Basbaum et al. 1978). The electrical stimulation in NRM produces inhibition of the discharge of the spinal cord nociceptive neurones (Fields et al. 1977b; Oliveras et al. 1977; Willis et al. 1977; Belcher et al. 1978). The nucleus locus coeruleus as has been described already projects to the spinal cord but has also been shown to have a morphologic linkage with the other brain-stem nuclei such as the nucleus raphe magnus (NRM). In order to find any contribution that NRM might make in mediating some of the LC actions, the effect of LC and NRM stimulation was tested and compared on the discharge of the same multireceptive neurones. The effects exerted from both the nuclei were qualitatively and in most cases quantitatively similar. The predominantly inhibitory effect of NRM has also been reported in other studies (Fields et al. 1977 b; Oliveras et al. 1977; Willis et al. 1977; Belcher et al. 1978; McCreery et al. 1979). The mixed excitation and inhibition or excitation alone observed in the present study was not reported in some studies (Willis et al. 1977; Gerhart et al. 1981) but similar effects produced by stimulation in NRM have been reported by other investigators (Fields et al. 1977 b; Belcher et al. 1978; McCreery et al. 1979). Although a selective inhibitory action on the noxious input has been observed to be produced by stimulation in LC and NRM, in the majority of the neurones a non-selective inhibitory action has been found on both the A and the C-fibre evoked discharge. However the inhibitory action seemed to be more pronounced on the discharge evoked by the Aδ and C-fibres. These findings are consistent with the findings of

other investigators (Willis et al. 1977; Gerhart et al. 1981) who also observed a pronounced inhibitory action exerted by NRM on the discharge evoked by the Aδ and C-fibres. However a lesser magnitude of inhibition was also produced on the earlier A-fibre evoked discharge (Willis et al. 1977; Gerhart et al. 1981). The effects produced from LC and NRM on the discharge of the same neurones do not indicate the involvement of a particular pathway or pathways, which required a detailed analysis that would be discussed further in the discussion relating to the pharmacological and the lesion studies conducted in the present investigation.

In order to study the involvement of catecholamines in the mediation of the locus coeruleus produced effects, various α- and β-receptor antagonists were administered intravenously. The evidence presented in the present investigation suggests that the inhibition of the discharge of multireceptive neurones produced from LC is mediated by the α-noradrenergic receptors whereas the β-receptors do not seem to play a part in the inhibition. The noradrenergic transmission mediating the locus coeruleus effect is consistent with the findings of other investigators (Sasa et al. 1974; Strahlendorf et al. 1980; Yakhnitsa and Pilyavsky, 1980 b). The inhibitory effect produced by the stimulation in the nucleus locus coeruleus on the spinal trigeminal neurones (Sasa et al. 1974) and on the extensor monosynaptic reflex (Yakhnitsa and pilyavsky, 1980 b) has been reported to be blocked on pretreatment with reserpine which has demonstrated the monoaminergic nature of the transmission involved in mediating LC actions. The locus coeruleus effect in the cerebellum, hippocampus and cortex however seems to be mediated by

the β-noradrenergic receptor possibly coupled to an adenylate cyclase (Moore and Bloom, 1979). In a recent study on the hippocampal slice, using the microdrop application of NA along the entire apical dendritic region has been observed to cause a hyperpolarisation but the effect was marked when NA was applied on the soma (Segal, 1981). It has been proposed that NA activates two mechanisms, one involving activation of Cl-conductance and the other activation of a Na+ - K+ pump which might be coupled to an adenylate cyclase. In the visual cortex the inhibitory effect produced by the electrical stimulation in LC or by the microiontophoretic application of NA was also shown to be mediated by the β-receptor (Olpe et al. 1980). However the involvement of α-receptor mediating the inhibition produced by NA has also been demonstrated in the cortex (Sharma, 1977). The results of the present investigation do not agree with the findings showing the involvement of the β-receptor in mediating the inhibition produced from LC or by NA applied microiontophoretically but instead provide evidence that in the spinal cord locus coeruleus produced inhibition involved α-receptors. These findings however are in agreement with the findings of Strahlendorf et al. (1980) who have demonstrated that the LC produced facilitation of the spinal cord monosynaptic reflexes is also mediated through the α-receptor.

There is further evidence available to support the involvement of the α-receptors in mediating the SPA and MA. The intrathecal administration of α-receptor antagonist phentolamine blocks the analgesia produced by the microinjection of morphine into the PAG in the rat (Yaksh, 1979). It has also been demonstrated that NA

applied intrathecally in the spinal cord produced analgesia which was also blocked by pretreatment with phenoxybenzamine, a α-receptor antagonist (Kuraishi et al. 1979b). These studies showed that the NA released from the descending noradrenergic fibres in the spinal cord produced analgesia which involved the participation of the α-receptor. The inhibitory effect of electrophoretically applied NA on the spinal cord neurones has been shown in several studies (Engberg and Ryall, 1966; Headley et al. 1978; Belcher et al. 1978). The microelectrophoretic application of NA and 5-HT in the SG produce a predominantly selective reduction in the nociceptive response of deeper dorsal horn neurones (Headley et al. 1978). The inhibitory and rarely an excitatory action of the microelectrophoretically applied NA near the cell bodies has also been observed on the spontaneous or the evoked response of nociceptive neurones in the spinal cord (Belcher et al. 1978). The inhibitory effect of NA was not antagonised by the α- and β-receptor antagonists (Belcher et al. 1978). Though the intravenously given drugs do not give any indication of the site or sites of action of a drug but the microiontophoretically applied drugs will also only produce an action if the application or the applied drug is near to the region of the appropriate active synapses. An antagonist applied near the cell body may not produce the expected action if the appropriate active synapses will be located on the distal dendrites. However in addition to the intravenous administration of antagonists, microiontophoretic application of agonists and antagonists in the present investigation would have helped to identify the mediators more positively.

The substantial evidence presented together with the demonstration of descending catecholamine projections to the dorsal and ventral horn of the spinal cord from the locus coeruleus (Nygren and Olson, 1977; Westlund and Coulter, 1980) provides the basis for speculating that the majority of the effects observed in the present study were presumably mediated by the descending NA containing fibres from LC which involved the participation of the α-receptors. The evidence presented on the current spread of less than 1 mm from the stimulated site together with the histological location of the sites of stimulation within the LC and the involvement of NA in mediating these actions provided sufficient information to suggest that the actions produced by the stimulation of the nucleus locus coeruleus are produced by the activation of elements in the LC. However gross stimuli can produce activation of nearby structures and can also excite the axons of passage. Some of these questions would be further resolved in the later parts of the discussion. It is interesting in this regard that the inhibitory action produced on reflex transmission from the surrounding structures in the reticular formation is not mediated by the monoaminergic transmission (Yakhnitsa and Pilyavsky, 1980 b).

It was originally suggested that the LC analgesia is mediated by the serotonergic transmission (Sandberg and Segal, 1978) but later work (Margalit and Segal, 1979) has shown that it involves catecholaminergic, serotonergic and opioid mechanisms. It has been shown that the depletion of catecholamines potentiates analgesia (Akil and Liebeskind, 1975) which seems paradoxical in view of the evidence presented so far. However tonically active inhibitory connections

of noradrenergic structures presumably with some nuclei which might be tonically suppressing the nociceptive transmission can explain these results. The depletion of a transmitter can produce wide ranging effects which cannot be easily explained.

In order to resolve the question of pathway or pathways involved and to further investigate the role of other putative transmitter substances which might be mediating LC actions that may also help to differentiate between the LC and NRM pathways, various antagonists for serotonin, opioids and GABA were used. These antagonists were used on the premise that if these putative transmitters are involved in mediating LC and NRM actions then an antagonism of the actions produced from these nuclei will be observed.

The inhibition produced by stimulation of the nuclei locus coeruleus and raphe magnus was not changed after the intravenous administration of 5-HT antagonists cinnanserin and methysergide. It has been proposed that methysergide is a specific antagonist of 5-HT actions in the central nervous system (Segal, 1976). The 5-HT antagonists were administered on the premise that actions of the 5-HT raphe spinal pathway would be blocked leaving the locus coeruleus actions unchanged which would indicate the significance of the direct 5-HT raphe spinal and the noradrenergic coeruleospinal pathways to the spinal cord, independently modulating the nociceptive transmission in the spinal cord. The failure to antagonise the NRM inhibition of the spinal cord multireceptive neurones by administering the serotonin antagonists is consistent with the findings of Belcher et al. (1978) who also did not observe any antagonism of the

inhibitory action produced on the spinal cord nociceptive neurones by stimulation in the same region. However methysergide did antagonise the excitation produced from the NRM stimulation and a similar antagonistic effect of methysergide applied electrophoretically has also been observed on the excitation produced either by 5-HT or by the stimulation in the nucleus raphe magnus (Belcher et al. 1978). In a recent study, methysergide either applied electrophoretically in the SG or given intravenously did not reduce the inhibition of spinal cord nociceptive neurones produced by the stimulation in the medullary raphe nuclei (Griersmith et al. 1981). However Griersmith and Duggan (1980) have observed an antagonism produced by methysergide on the prolonged inhibitory effect of the nociceptive transmission produced by the electrophoretically applied 5-HT in the SG. It has been reported (Proudfit et al. 1980) that the long latency dorsal root potentials evoked from the stimulation in the caudal raphe nuclei were attenuated by systemically given methysergide and cinnanserin. It should be pointed out that the long latency dorsal root potentials were never evoked (Mokha et al. - unpublished obervations) from the stimulation in the NRM and the short latency DRPs were never attenuated by the 5-HT antagonists. It is however possible that long latency DRPs observed by Proudfit et al. (1980) can be evoked only in the decerebrate preparation. However consistent with our findings are the results of Azami et al. (1979) who did not observe attenutation of DRPs evoked from the same brain-stem sites in the rat after the administration of methysergide and cinnanserin. It has been reported that LSD blocked the dorsal raphe produced inhibition of the nociceptive transmission in the spinal cord (Guilbaud et al. 1973). However LSD is also known to

be a partial agonist. LSD and 5-HT have been demonstrated to produce depression of the firing of the serotonin containing neurones in the midbrain raphe region (Aghajanian et al. 1972). It has been shown recently from the receptor binding studies that two types of serotonin receptors exist in the CNS, the agonists display high affinity for the receptor 5-HT_1, and the antagonists prefer 5-HT_2 (Peroutka and Snyder, 1981). The levels of these two receptors have been shown to vary in different regions of the brain. Although the distribution of these receptors in the spinal cord is not known as yet, perhaps the 5-HT_2 receptors exist in low levels. It is also possible that from the midline raphe complex descending fibres contain other transmitter substances such as substance-P and Met-enkephalin (Hökfelt et al. 1980) or perhaps more specific and potent antagonists for the 5-HT receptors are required. There has been some evidence provided by Lundberg's laboratory (Engberg et al. 1968 b) on the involvement of serotonin in the tonic descending control of transmission from the flexor reflex afferents (Group II and III afferents). The serotonin antagonist (BOL) given systemically, increased the inhibition produced by the flexor reflex afferents in the extensor reflex indicating facilitation of transmission at an interneuronal level in the pathway from the flexor reflex afferents. These findings become paradoxical seen in the light of the recent evidence which suggests the non-involvement of 5-HT in the tonic descending control of the discharge evoked by the noxious cutaneous stimulation or by volleys in the C-fibres. However there are obvious differences like the decerebrated preparation (Engberg et al. 1968 b) and an anaesthetized reversibly spinalized preparation (Griersmith et al. 1981). An enhanced tonic descending inhibition

would be expected in a decerebrated preparation which is perhaps mediated by the release of 5-HT from the descending fibres or otherwise it could be a selective phenomenon acting only on flexor reflex afferent pathways and not on the C-fibres or on interneurones in the C-fibre pathways. In this regard it is also interesting that inhibition from medullary raphe nuclei of monosynaptic short latency reflexes was antagonised by methysergide whereas it did not affect the inhibition of polysynaptic reflexes (Clineschmit and Anderson, 1971) and it is the polysynaptic spinal reflexes which are presumably more important for the spinal nociceptive transmission. Similarly the findings of Proudfit et al. (1980) mentioned earlier about the raphe evoked long latency DRPs being reduced by methysergide and cinnanserin, also showed that the long latency DRP was presumably generated by the depolarisation in the group I afferents.

The partial reduction in the LC produced inhibitory effect was observed after naloxone administration suggesting that opioids might play some part in mediating LC actions which is consistent with the findings of Margalit and Segal (1979) showing that analgesia produced from LC was attenuated by naloxone. On the other hand, although the analgesia produced from the nucleus inferior centralis raphe has been shown to be reduced by naloxone (Oliveras et al. 1977) yet in the present investigation the NRM evoked inhibition of nociception transmission was not changed by naloxone administration. However on a few instances reduction in the inhibition or attenuation in the earlier part of complete inhibition combined with an enhanced inhibition in the period subsequent to that has been observed on a superficially located (lamina I) multireceptive neurone. The descending coeruleo-

spinal fibres in addition to catecholamines may also contain other putative transmitter substances such as neurotensin, substance-P or a peptide similar to an avian pancreatic polypeptide which has been shown recently to co-exist in the catecholamine containing neurones in the nucleus locus coeruleus (Hunt et al. 1981). Even if the descending coeruleospinal fibres do not contain a peptide whose actions can be antagonised by naloxone, enkephalin-containing interneurones, particularly in the SG, can mediate some of the actions observed. The inhibition of spinal nociceptive transmission by intravenous morphine has been shown to be antagonised by naloxone given systemically (Section III) or administered in the substantia gelatinosa (Johnson and Duggan, 1981). Similarly opioid peptides applied in the SG have been shown to cause a selective reduction in the nociceptive discharge of multireceptive neurones (Duggan et al. 1977 a, b). In a decerebrated spinalized rat it has been demonstrated that naloxone caused excitation of C-fibre discharge of neurones in laminae IV and V and inhibited the discharge of neurones lying in lamina I and II (Fitzgerald and Woolf, 1980). Thus it seems that opioids cause inhibition of the deeper neurones whereas excitation is observed in the SG region. Opioids may cause excitation in SG by disinhibition as suggested for the excitation observed in the hippocampus (Zieglgänsberger et al. 1979) or perhaps the hyperpolarisation of primary afferents by opioid peptides indicated in several studies (Jessel and Iversen, 1977; Sastry, 1979) cause presynaptic facilitation of the C-fibre evoked discharge in the enkephalin containing interneurones in the SG, which on activation or due to their background discharge may exert an inhibitory control on the deeper neurones. The enkephalin-containing interneurones

linked monosynaptically to the C-fibres can cause hyperpolarisation of the C-fibre terminals through dendro-axonic synapses. There is evidence to show the existence of the dendritic release of dopamine in the substantia nigra (Glowinsky et al. 1981) and recently dendro-axonic synapses have been described in the substantia gelatinosa (Gobel et al. 1980). Naloxone given systemically could also act by acting at the axo-dendritic synapses in the LC which has been shown to exist between the Met-enkephalin containing nerve terminals and dendrites of the catecholamine neurones (Pickel et al. 1979). The inhibitory effect of morphine, GABA and NA has been shown on coeruleospinal neurones (Guyenet, 1980). The cells in the locus have also been reported to be inhibited by Met-enkephalin (Guyenet and Aghajanian, 1977; Pepper and Henderson, G, 1980) and by β-endorphin (Strahlendorf et al. 1980 a).

It is suggested, based purely on speculation, that LC produced excitation could be produced by NA, neurotensin or substance-P present in the descending coeruleospinal terminals. Neurotensin and substance-P are present in neurones in the LC presumably in the same neurones containing NA or in separate neurones and these two peptides can mediate the excitation in the dorsal horn as both are known to produce excitation of spinal cord nociceptive neurones. (Miletic and Randić, 1979; Henry et al. 1980). However neurotensin has also been shown to cause prolonged inhibition of neurones in the ventrobasal thalamus in the rat (Dao and Walker, 1980).

GABA antagonist bicuculline abolished the DRPs evoked from LC and NRM as well as the inhibition of the multireceptive neurones

indicating the role of GABA in mediating the descending effects from the brain-stem. The role of GABA will presumably be at an interneuronal level; a GABA containing interneurone interposed in the pathways from LC and NRM. The dorsal part of the dorsal horn is known to contain high levels of GABA, (Otsuka and Konish, 1976) and glutamate decarboxylase activity. The existence of axo-axonic synapses in this region indicate the potential role that GABA containing interneurones might play in producing pre- and postsynaptic inhibition in the spinal cord. The role of GABA in the presynaptic inhibition was proposed by Eccles et al. (1963) and from the later studies it was proposed that GABA is the transmitter at synapses which are sensitive to bicuculline or picrotoxin but are insensitive to strychnine (Curtis et al. 1971). The ability of bicuculline and strychnine to differentiate between the GABA and glycine receptors has been demonstrated in the rat spinal cord but these antagonists do not seem to be able to consistently differentiate between the inhibitory amino acids in the cerebral cortex (Biscoe et al. 1972). The early short duration and the late prolonged inhibition of dorsal horn neurones produced by volleys in mixed myelinated cutaneous afferents have been shown to be mediated by glycine and GABA respectively (Game and Lodge, 1975). The antagonism of inhibition evoked from LC and NRM does not seem to be due to a non-specific effect because in the same experiments although bicuculline abolished the evoked DRPs yet it did not abolish the spontaneous DRPs (Mokha et al. - unpublished observations). Bicuculline, sometimes, did produce a changed pattern of neuronal firing, normal regular firing to a bursting pattern. Since bicuculline also abolished the segmental DRPs it is reasonable to conclude that the site of action of bicucu-

lline in the present study was in the spinal cord. This interpretation perhaps should be exercised with a caution which is necessary in view of the finding that GABA containing nerve terminals do exist in the LC. However in spite of that it is still likely that the action was in the spinal cord because the GABA agonist muscimol injected into the LC has been reported to cause increased firing rate in the lumbar spinal cord neurones (Benelli et al. 1979) and the action of bicuculline in the LC itself should, if at all, produce enhanced inhibition. Some evidence has been provided recently showing that GABA and glycine are not involved in the tonic descending control of the nociceptive transmission in the spinal cord in the cat (Duggan et al. 1981).

The present investigation also provides evidence that the locus coeruleus modulation of the spinal cord nociceptive transmission is not mediated through a relay in the NRM and is most likely to be mediated through the direct coeruleospinal projections. The selective lesions placed in the various quadrants of the spinal cord demonstrated the pathway mediating locus coeruleus actions located mainly in the ipsilateral ventrolateral and ventral funiculi whereas the NRM effects seemed to be mediated through a pathway located bilaterally in the DLF. The pathway mediating the LC modulation of the spinal sensory transmission being located in the ventrolateral and ventral funiculi is consistent with the anatomical evidence. Based on the retrograde transport of HRP the descending pathway from the locus coeruleus has been shown to be located mainly in the ventrolateral funiculus (Kuypers and Maisky, 1977; Tohyama et al. 1979; Westlund and Coulter, 1980). A small projection through the DLF and

VF has also been shown in these studies (Kuypers and Maisky, 1977; Tohyama et al. 1979). The coeruleospinal projection from LC in the monkey has been shown to be ipsilateral and changes its mainly ventral position (VLF and VF) in the cervical and thoracic regions to a slightly dorsal position in the lumbar region (Westlund and Coulter, 1980). Some of the axons at the sacral level cross to the other side of the cord into the DLF.

Retrograde HRP studies have revealed a few cells labelled, if at all, in the LC contralateral to the site of injection in the spinal cord (Tohyama et al. 1979). However using retrograde transport of the active and inactive form of HRP Hayes and Rustioni (1981) found cells labelled in the LC on both sides, on making an ipsilateral injection in the lumbar spinal cord in the cat. These observations on the surface seem to differ from the evidence provided by other investigators (Kuypers and Maisky, 1977; Tohyama et al. 1979) but looking carefully at that study (Hayes and Rustioni, 1981) shows that it is consistent with the findings of other investigators. In that particular study the HRP injections involved the ipsilateral gray matter which also included part of the gray matter on the contralateral side. From the evidence provided from their study it is suggested here that the ipsilaterally descending coeruleospinal fibres send massive collaterals from the ipsilateral gray matter to innervate the gray matter on the contralateral side which can explain the results obtained in that study. This suggestion is supported by the recent findings (Westlund and Coulter, 1980) demonstrating the terminals innervating both sides of the gray matter though the descending LC axons remain in the ipsilateral ventrolateral quadrant,

at least, up to the level of the lumbosacral enlargement. The other investigations did not find cells labelled in LC on both sides since the injections were made in the white matter on one side of the cord. However a bilaterally located pathway from the SC-PB complex have been demonstrated in the monkey (Westlund and Coulter, 1980). In the rat however evidence for both the crossed and uncrossed projections from LC to the spinal cord exists (Karoum et al. 1980; Commissiong, 1981). The ventrolaterally located pathway from locus coeruleus in the rat, innervating the ventral horn, intermediate gray and dorsal half of the dorsal horn have been demonstrated by fluorescent histochemistry (Nygren and Olson, 1977). The findings of Westlund and Coulter (1980) in the monkey showing innnervation of laminae I-IX excepting lamina III corresponds very well with the evidence in the rat (Nygren and Olson, 1977). A partial contribution of noradrenergic nerve terminals in the superficial dorsal horn originating from LC was also observed in the study of Nygren and Olson (1977) though the remaining innervations have been suggested to be from the cell bodies lying in areas A_1-A_3 which are known to project to the spinal cord (Dahlström and Fuxe, 1965).

The nucleus raphe magnus (NRM) modulation of the spinal cord nociceptive transmission was observed to be mediated through a pathway located mainly in the DLF bilaterally. This is consistent with the findings of other investigators providing evidence that the raphe spinal pathway is located bilaterally in the DLF (Fields et al. 1977 b; Basbaum et al. 1978; Martin et al. 1979). However sometimes even a bilateral DLF lesion failed to block the effects from NRM in the present study indicating that DLF is not the only

area containing descending raphe spinal axons. It is interesting in this regard that even a bilateral DLF lesion in the monkey sometimes did not completely abolish the primary afferent depolarisation evoked from NRM (Martin et al. 1979). A small moderate projection through VLF from NRM has been demonstrated (Kuypers and Maisky, 1977; Tohyama et al. 1979). The 5-HT axons innervating the spinal cord from the caudal raphe nuclei have also been shown to be present in the dorsal and the ventral quadrant of the spinal cord (Dahlström and Fuxe, 1965).

The extensive electrolytic lesions made in the raphe nuclei which included almost complete destruction of NRM also did not abolish the locus coeruleus stimulation-produced modulation of the spinal nociceptive transmission. A caution is necessary to be exercised here since electrolytic lesions do not necessarily mean that all the serotonin containing neurones in the raphe complex have been destroyed and in that respect selective chemical lesions are the best way to approach such a problem as that. However because of the recent demonstrations of substance-P and Met-enkephalin presence in this area, the electrolytic lesions are perhaps better suited for the purpose than the chemical lesions.

Although Sakai et al. (1979) have demonstrated a moderate projection from the LCα to the NRM and there is also evidence to show the afferent and efferent connections between LC and the raphe complex (Amaral and Sinnamon, 1977; Chu and Bloom, 1974; Segal, 1979; Watabe, 1980) yet there are studies that show that in the rat the LC does not contribute much to the noradrenergic innervation of

NRM. The electrolytic lesions placed in the LC have been shown to cause a reduction in the content of NA in the NRM but the terminal density was found unchanged (Levitt and Moore, 1979). However it is quite possible that the terminal density of the NRM was observed to be the same which might have been obsecured by the brightly fluorescent thick fibres of the lateral tegmental origin (Levitt and Moore, 1979). A small labelling was observed in LC on injecting HRP in the NRM (Gallager and Pert, 1978; Sakai et al. 1979). Lesions made by monosodium glutamate in the LC have been shown to decrease the NA content in NRM (Hammond and Proudfit, 1980) and recently using the anterograde autoradiographic technique (Westlund and Coulter, 1980) it has been demonstrated that the noradrenergic nerve terminals of LC origin innervate the NRM in the monkey.

Microinjection of noradrenergic α-receptor antagonist (phentolamine) into NRM has been recently shown to produce hypoalgesia in the rat (Hammond et al. 1980). This evidence provides an indication that perhaps the tonically active descending raphe spinal neurones are under a tonically active noradrenergic inhibitory control exerted presumably from the LC.

The evidence presented in the present investigation suggests that the locus coerueleus modulation of spinal nociceptive transmission is possibly mediated through the direct coeruleospinal projections which are anatomically well defined (Kuypers and Maisky, 1975, 1977; Hancock and Fougerousse, 1976; Nygren and Olson, 1977; Tohyama et al. 1979; Basbaum and Fields, 1979; Westlund and Coulter, 1980; Hayes and Rustioni, 1981). However a caution should be

exercised here since there could be small subtle interactions between LC and NRM or other nuclei such as the nucleus reticularis magnocellularis (RMc) which may contribute, if at all, a very small part in mediating LC actions. Similarly the nucleus raphe magnus is suggested to exert its influence on the spinal nociceptive transmission through the raphe spinal projections which is consistent with the findings of other investigators (Fields et al. 1977 b; Basbaum et al. 1978; Fields and Anderson, 1978; Tohyama et al. 1979).

The discussion from now on will look at the pre- and post-synaptic mechanisms involved in the descending modulation of the spinal nociceptive transmission from the nucleus locus coeruleus. The results presented in the present investigation indicate the involvement of both the presynaptic and the postsynaptic mechanisms. A selective presynaptic inhibitory control on the nociceptive transmission combined with the postsynaptic excitation can also explain some of the results obtained in the present study. However since IPSPs produced from LC were recorded from a few neurones and a non-selective inhibitory effect was also observed from LC, the post-synaptic inhibitory mechanisms are also suggested to be involved in mediating these actions. However the postsynaptic inhibitory mechanism itself can also produce a selective inhibitory action if its action is postulated on the interneurones in the pathway from nociceptive afferents only or else a remote dendritic inhibition can also be expected to produce a similar selective inhibitory action. It has been reported (Engberg et al. 1968 d) that IPSPs were produced in five out of a large population of intracellularly recorded neurones on stimulation of the caudal brain-stem, corresponding to

Magoun's inhibitory centre. The pathway mediating these effects was described as the dorsal reticulospinal system (Engberg et al. 1968 c) and the five neurones on which IPSPs were evoked were reported to have monosynaptic connections from the periphery (Engberg et al. 1968 d). In the present investigation stimulation of an area corresponding to Magoun's inhibitory centre (nucleus reticularis magnocellularis) produced an IPSP in a multireceptive neurone and on the same neurone IPSP evoked from LC was also observed. In majority of the interneurones (Engberg et al. 1968 d) stimulation of the caudal brain-stem did not produce any post-synaptic potentials but effectively depressed the EPSPs as well as the IPSPs evoked by the flexor reflex afferents. These inhibitory effects (Engberg et al. 1968 d) were observed using current intensities that did not produce DRPs. However in the present investigation the threshold current intensities that produced inhibition also evoked DRPs from both the LC and NRM providing additional evidence for the involvement of the presynaptic inhibitory mechanism.

At this stage the morphology of the synaptic contacts that the descending noradrenergic nerve terminals make should be discussed which may shed some light to explain some of the findings. The only information available on the ultrastructure of the synaptic contacts made by the descending noradrenergic nerve terminals is in the nucleus tracts spinalis nervi trigemini. In a combined histofluorescent, retrograde HRP and electron microscopic study (Senba et al. 1981) have demonstrated that the noradrenergic nerve terminals from LC and area A_1 in the rat were observed to establish predominantly axo-dendritic synapses on the proximal dendrites but axo-axonic synapses

were also present. However, surprisingly, in the axo-axonic synapses (Senba et al. 1981) the noradrenergic nerve terminal was always observed to be postsynaptic to the other non-noradrenergic nerve terminal belonging either to the primary afferents or to descending axons from other places. The plug cleft synapses were also observed in which a single dendrite in the spinal trigeminal nucleus received one noradrenergic and one non-noradrenergic nerve terminal and these two terminals were also observed to establish axo-axonic synapses. Occasionally a noradrenergic nerve terminal has been seen to establish contacts with several dendrites (Senba et al. 1981). If a similar synaptic situation is speculated for the descending noradrenergic nerve terminals in the spinal cord establishing axo-axonic and axo-dendric synapses with the neuronal perikaria in the lumbar spinal cord, one can imagine the involvement of both the pre and postsynaptic mechanisms. It is intriguing however that the descending noradrenergic nerve terminal should be postsynaptic to another non-noradrenergic nerve terminal possibly belonging to other descending pathways. This raises the possibility that descending pathways may modulate the activity of each other by changing the level of depolarisation in the region of terminals. However perhaps the terminals of primary afferents or other interneurones possess this speculative ability as well. It has been suggested recently by Gobel et al. (1980) that axonal endings having similar ultrastructural characteristics to the 5-HT and NA containing nerve terminals were observed to establish synapses on the dendrites of the intracellularly stained stalked or islet cells in the SG. It is known that the noradrenergic nerve terminals do not necessarily show characteristics of a typical synapse. The axo-axonic contacts

made by LC nerve terminals have been observed less frequently in the hippocampus, cerebellum, cerebral cortex and hypothalamus (Moore and Bloom, 1979). Not only does the locus coeruleus axon break up into an ascending and descending branch innervating several areas of the neuroaxis but also the pre-terminal fibre breaks into a highly collateralised network (Moore and Bloom, 1979). An exhaustive ultrastructural analysis of the noradrenergic nerve terminals (Descarries et al. 1977) revealed that only 5% of the labelled terminals made synaptic contacts in the cerebral cortex. This finding raises interesting possibilities of the highly plastic nature of the noradrenergic nerve terminals which perhaps continue growing and making new contacts. This would mean that electrical stimulation of LC causing the release of NA will possibly be acting on the nearby postsynaptic elements having noradrenergic receptors. This also raises the question that perhaps the definition or the criteria of a synapse defined morphologically is too restrictive to exclude certain functional synapses.

The morphological evidence provides interesting possibilities and provides support for the suggestion that the locus coeruleus stimulation produced effects on the multireceptive neurones would most probably involve both the pre- and the postsynaptic mechanisms. Some evidence regarding this has been provided in the present investigation. However there could be differences in the morphology of these synapses in the various regions of the CNS and there could be variations in different species as well. Axo-dendritic synapses formed by LC terminals located more proximal to the cell soma than

the excitatory axo-dendritic synapses formed by Aδ and C-fibres can also produce selective inhibitory action. Bicuculline has been the only drug that abolished the DRPs evoked segmentally and by the descending pathways from LC and NRM whereas the other drugs such as methysergide, cinnanserin (5-HT antagonists) and naloxone did not block these DRPs. The inhibition by bicuculline of evoked DRPs seemed to be fairly selective since the spontaneous DRPs were not changed (Mokha et al. - unpublished observations). However there has been a report in the decerebrated cat that serotonin antagonists abolished the long latency evoked DRP from NRM (Proudfit et al. 1980). In the present investigations no long latency DRPs as described by Proudfit et al. (1980) were observed. The long latency DRPs could have been produced in that study because of the release of the brain stem from the cortical inhibitory control. Carstens and Zimmermann (1981) have reported hyperpolarisation of Aδ and C-fibre terminals produced by microiontophoretically applied 5-HT which does not fit with the current concepts of pre-synaptic inhibition. Morphine and enkephalins have been shown to hyperpolarise Aδ and C-fibres (Sastry, 1979; Carstens and Zimmermann, 1981). It is suggested that in the generation of segmental as well as supraspinal DRPs from LC and NRM there does not seem to be an involvement of opioid mechanisms which is consistent with the findings of Sastry (1979). Morphine has been shown to inhibit the K+ evoked release of substance-P in the trigeminal slice (Jessel and Iversen, 1977) which though described as due to presynaptic inhibition yet shows that morphine must have produced hyperpolarisation of primary afferents, on the assumption that the K+ evoked release reflected a synaptic mechanism.

The observation that the threshold current intensities producing inhibition evoked DRPs and that bicuculline abolished both does indicate the role of presynaptic inhibition. However these findings can also be interpreted that bicuculline inhibited the inhibitory interneurone in the pathway from LC and NRM thus producing antagonism of this inhibition and also abolished DRPs by its action on a different group of GABA containing interneurones that produced the DRPs. This possibility however does not seem very convincing.

How these findings relate to the findings of others and if there is such a phenomenon as the pre-synaptic inhibition will be discussed here. The stimulation of the nucleus raphe magnus in the monkey (Martin et al. 1979) has been reported to produce depolarisation of Aα, β, and Aδ fibres (Martin et al. 1979) and the stimulation in NRM has also been shown to produce IPSPs (Giesler et al. 1981) whereas in the cat higher intraspinal threshold for C-fibres has been observed (Hental and Fields, 1979). The concept of primary afferent depolarisation producing inhibition (Schmidt, 1971) becomes paradoxical in the light of the evidence provided by Singer and Lux (1973). The stimulation of the mesencephalic reticular formation caused a prolonged increase in the excitability of the optic tract fibres in the lateral geniculate nucleus but intracellular recordings did not reveal any reduction in the amplitude of the evoked EPSPs and in fact a facilitation of transmission was observed (Singer and Lux, 1973; Singer, 1973). It is of course possible that higher levels of K+ released by the activated neurones and axons can account for the depolarisation (Singer and Lux, 1973) which may be consequential to the activation and thus perform no physiological function.

However the demonstration of axo-axonic synapses in the spinal cord combined with the studies that indicate the existence of PAD does suggest that in the spinal cord a presynaptic inhibitory mechanism exists. It has already been described that morphine, enkephalins and 5-HT have been reported to produce hyperpolarisation in the Aδ and C-fibre terminals. The role of GABA in presynaptic inhibition is widely accepted and evidence has been provided that microiontophoretically applied GABA causes depolarisation in the Group I afferent terminals (Curtis et al. 1977; Curtis and Lodge, 1978). Recently it has been reported that GABA increased the terminal excitability of C-fibres in the majority of fibres tested whereas both increased and decreased terminal excitability was observed for Aδ fibres (Randić, 1981). The question arises as to why the inhibition cannot be generated by a remote dendritic form of inhibition. The evidence for the presynaptic inhibition rests mainly on the evidence that there is no change in the time course of the EPSP during presynaptic inhibition and it is only the amplitude of the EPSP which is reduced, caused by the reduced amount of the transmitter being released as a consequence of the depolarisation of the primary afferent nerve terminals (Eccles et al. 1961; Hubbard et al. 1967; Schmidt, 1971). The presynaptic inhibition is based on the assumption that the decay phase of the EPSP is caused by excitation at the remote dendritic synapses but the time course of the transmitter action can perhaps play a part. Since the present investigation dealt mainly with the modulation of the spinal sensory transmission it is important that other aspects of the synaptic distribution should also be discussed. Axo-somatic synapses both excitatory and inhibitory, located near the axon hillock would be effective in

producing excitation or inhibition since depolarisation or hyperpolarisation has to propagate electrotonically to the site of the impulse initiation. It is particularly advantageous for the inhibitory synapses to be located near the site of impulse initiation since the driving potential for the IPSP is small and would be reduced further in electrotonic conduction (Hubbard et al. 1969). Though it seems reasonable to have excitatory axo-somatic synapses, the inhibitory axo-somatic synapses formed by descending pathways would not be able to exert a fine control as would be expected based on the evidence of the present as well as several other investigations on the descending control of the sensory transmission. The dendritic form of inhibition (Burke et al. 1968) would however be very effective in controlling the dendritic input to the neurones (Rall, 1967) which can be expected to exert a fine control. It has also been suggested that the synapses situated at the dendrites can cause long lasting excitation (Tsukahara and Kosaka, 1966) and inhibition (Llinaś and Terzuolo, 1965). Thus it appears that the prolonged inhibition observed from LC can be mediated by the axo-dendritic synapses formed by the descending noradrenergic nerve terminals from LC (Senba et al. 1981) which can produce selective and non-selective inhibitory actions.

It is suggested from the evidence provided in the present investigation, seen in the light of the existing knowledge, that the inhibition produced by the descending axons from LC can exert an inhibitory control on the nociceptive transmission in the spinal cord presumably involving postsynaptic inhibitory mechanism as well as presynaptic inhibition. The postsynaptic inhibitory action can be

exerted through the axo-dendritic or perhaps through the axo-somatic synapses as well. The selective inhibitory action on the nociceptive transmission can be produced either by presynaptic inhibition or postsynaptic inhibition at an interneuronal level in the polysynaptic pathways from the high threshold cutaneous afferents to the deeper laminae. A remote dendritic form of inhibition could also contribute. However further detailed studies are required to elucidate the question of mechanisms involved; how monosynaptic and polysynaptic evoked synaptic activity is modulated by LC and NRM stimulation combined with simultaneous monitoring of the excitability of the primary afferents will be a daunting but feasible task to attempt to answer this question.

The substantia gelatinosa has been hypothetized to be involved in the modulation of sensory transmission in the spinal cord (Melzack and Wall, 1965; Cervero and Iggo, 1978). It has been shown recently that the SG is not a homogeneous entity and it contains different types of neurone. In a few instances, so far, recordings made in the superficial laminae (I and II) also revealed a predominantly inhibitory action on these neurones similar to the action observed on lamina V neurones. However the descending LC axons establishing axo-dendritic contact with the SG neurones can however be imagined to widen the scope of descending modulation that can be exerted. These contacts can be expected to extend the effect of LC stimulation several segments rostral and caudal to its entry through SG interneurones projecting into the Lissauer's tract. Similarly the commissural substantia gelatinosa neurones can be expected to mediate the actions of ipsilaterally descending pathways on the contralateral

side. The LC-LC interaction could also be important in the descending control. All these are speculative but testable possibilities.

It is proposed, based on the evidence provided, that the nucleus locus coeruleus produces the modulation of the spinal cord nociceptive transmission through direct coeruleospinal projections which is suggested to be the underlying neural mechanism of the analgesia produced from LC. Since there is evidence that suggests the reciprocal innervation of LC and dorsal raphe (Segal, 1979; Watabe, 1980) it is also suggested that the analgesia produced from dorsal raphe is mediated through a relay in the nucleus locus coeruleus. There has been no evidence so far of the projections to the spinal cord excepting a recent report from Jouvet's laboratory (Tohyama et al. 1979) that has provided evidence of the spinal cord projections from the dorsal raphe but only up to the cervical levels of the spinal cord in the cat. The nucleus raphe magnus (NRM) may play a very small subtle part, if at all, in mediating LC descending actions but the possibility of RMc playing a part is not excluded. However this latter possibility is not very strong. The locus coeruleus effects are mediated through noradrenergic α-receptors and there is also an indication of the partial involvement of opiates and GABA in mediating these effects, presumably at an interneuronal level in the spinal cord. Serotonin may be involved but the antagonists failed to provide any answer. The postural atonia indicated by the disappearance of EMG from the neck muscle observed in the paradoxical sleep (PS) has been suggested to be maintained by the nucleus locus coeruleus through a relay in the nucleus reticularis magnocellularis (Jouvet, 1979). The bilateral lesions placed in the

locus coeruleus (α) and its pathway does not affect the oneiric behaviour in the cat but the postural atonia associated with the paradoxical sleep (PS) disappears and the cat on entering PS starts moving its head as if watching something (Jouvet, 1979). The activity of neurones in the LC has also been observed to increase during the rapid eye movement (REM) stage (Chu and Bloom, 1973). The inhibitory effect of locus coeruleus stimulation exerted on cells in the ventral horn (Mokha, Iggo, McMillan - unpublished observations) will suggest that the postural atonia seen during the paradoxical sleep is also maintained most probably through the direct inhibitory coeruleospinal projections to the ventral horn.

The neurones in the locus are known to receive heterosensory input from tooth pulp, optic chiasm and sciatic nerve (Igarashi et al. 1979) and are also known to respond only to noxious stimuli (Moore and Bloom, 1979; Segal, 1979). There appears to be a feed-back loop between the locus coeruleus and the spinal cord, noxious stimuli exciting the neurones in the locus coeruleus which on activation would inhibit the discharge of the nociceptive transmission in the spinal cord. The cutaneous noxious messages to the locus coeruleus can be transmitted through the collaterals of the spino-thalamic tract neurones or else the spinoreticular tract through its relay in the reticular formation can excite the neurones in the LC. This information can however reach the LC from other levels of the neuraxis as well such as the thalamic nuclei.

There could be complex myriad of interactions in the LC because of its reciprocal connections with several areas in the

central nervous system which may all interact to influence the modulation of the sensory transmission along the ascending somaesthetic pathways, in the thalamus and in the sensory cortex. Here is a structure, the LC, which can be best described as a conductor keeping a watch over several areas of the neuraxis (the players) and directing the appropriate players (parts) so that a harmonious music would be produced (harmony in the CNS).

The conductor (LC) still conducts even after an orchestral play (awake state) has come to an end and most of the players perhaps rest (sleep). Here is a structure that with its complex interactions with the association cortex (memory) and the limbic system (emotions) yet to be revealed, would help to resolve some unresolved phenomenon.

FIGURE 4.1

The effect of stimulation in the locus coeruleus on the background discharge of a multireceptive neurones

Figures (A to E) show the recruitment of inhibition from LC and also from NRM. The records were taken from the same unit. LC was stimulated with 200 μ sec pulses of 300 μA for 80 m sec (except where noted). The interpulse interval in the train was 5 m sec (200 HZ).

A. The effect of changing the frequency of stimulation (repetition rate) of a train of stimuli (80 m sec at 200 HZ) at 300 μA.

B. The graph shows the quantitative data on the effect of increasing the repetition rate of a train of stimuli (80 m sec duration at 200 HZ).

C. The graph shows the recruitment of inhibition produced by increasing the length of the train from 5 m sec to 80 m sec, Other parameters were kept constant (200 μ sec pulses, 300 μA, 200 HZ frequency, train frequency 2/sec).

D;E. Recruitment of inhibition from LC and NRM on increasing the intensity of stimulation from LC (D) and NRM (E). The train of stimuli was repeated twice a second.

F. This record shows that on using similar stimulation parameters as mentioned above through an electrode lying approximately 1 mm medial to the electrode that produced the inhibitory effects (A-D) did not produce any inhibitory action on this neurone. Such sites are represented by open triangles in Figure 4.11.

Each point in the graph (B-E) represents a mean of 4 trials and the standard errors are marked as shown at each point.

Unit 1060-4

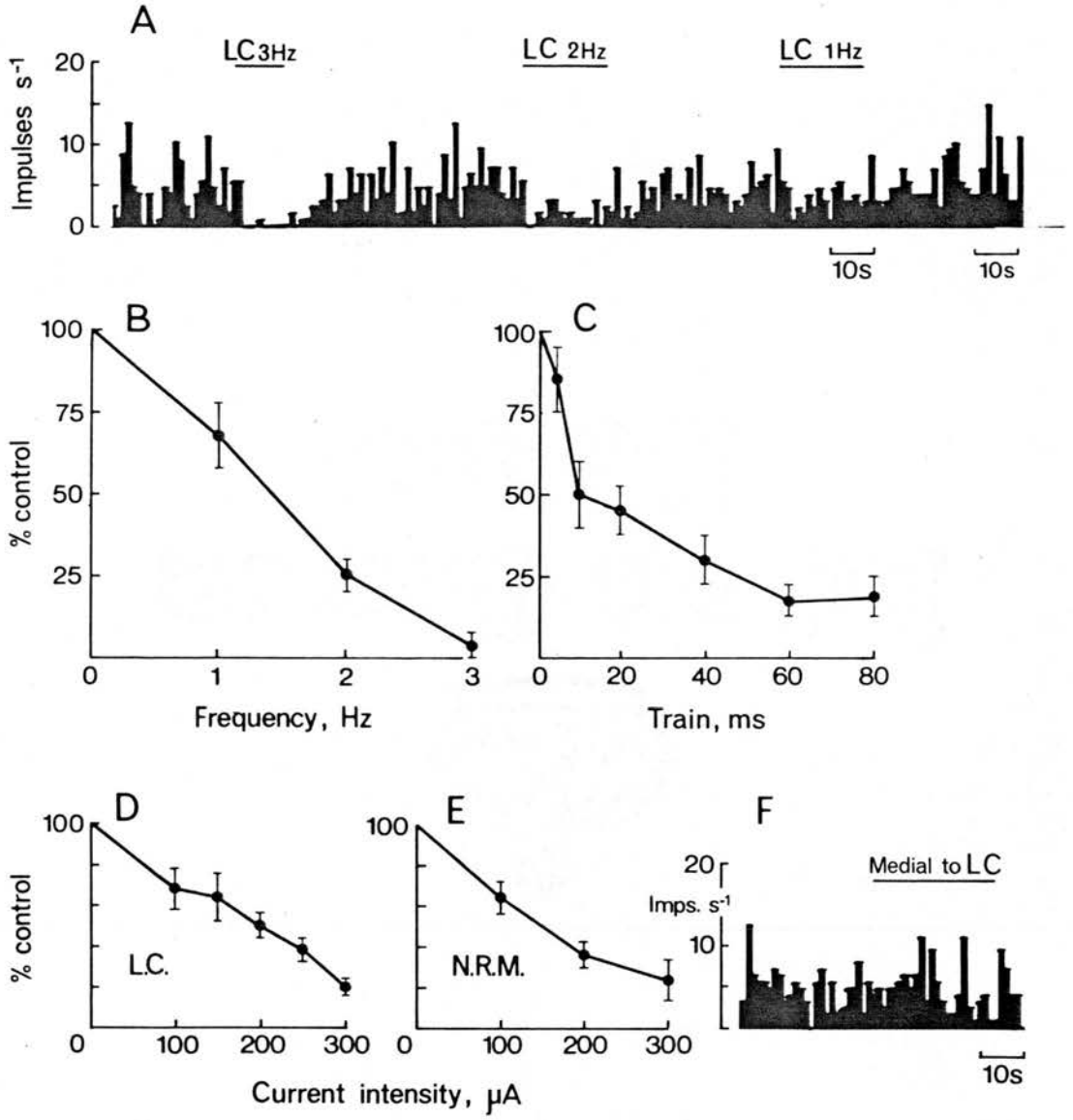

A
LC3Hz
LC 2Hz
LC 1Hz
Impulses s-1
20
10
0
10s
10s
B
100
75
50
25
% control
0
1
2
3
Frequency, Hz
C
100
75
50
25
0
20
40
60
80
Train, ms
D
100
% control
L.C.
0
100
200
300
E
100
N.R.M.
0
100
200
300
Current intensity, µA
F
20
Imps. s-1
10
Medial to LC
10s

FIGURE 4.2

Effects of locus coeruleus stimulation on background discharge

A. The effect of stimulation in the LC on the background discharge of this multireceptive neurone is shown in the frequency histograms. The bar on top represents the duration for which the locus coeruleus was stimulated at 300 μA, (30 m sec train at 500 HZ repeated every second).

B. The graph illustrates the recruitment of inhibition on the same multireceptive neurone shown in A. The current intensity was increased while the other parameters were kept the same. Each point represents the mean of 3 observations and the standard errors are plotted as shown.

C. The excitatory effect of locus coeruleus stimulation on another neurone when similar parameters of electrical stimulation were used are illustrated here. This particular unit was located in lamina VII and in addition to noxious and non-noxious cutaneous inputs also had muscle input. The threshold for producing excitation was >70 μA when a 5 ms train of stimuli (1000 HZ, 300 μA) was given at a frequency of 1 HZ. The minimum conduction velocity based on the variable latency of the first spike showed a conduction velocity of 28-40 m/sec assuming that the connection between the LC and this neurone was monosynaptic.

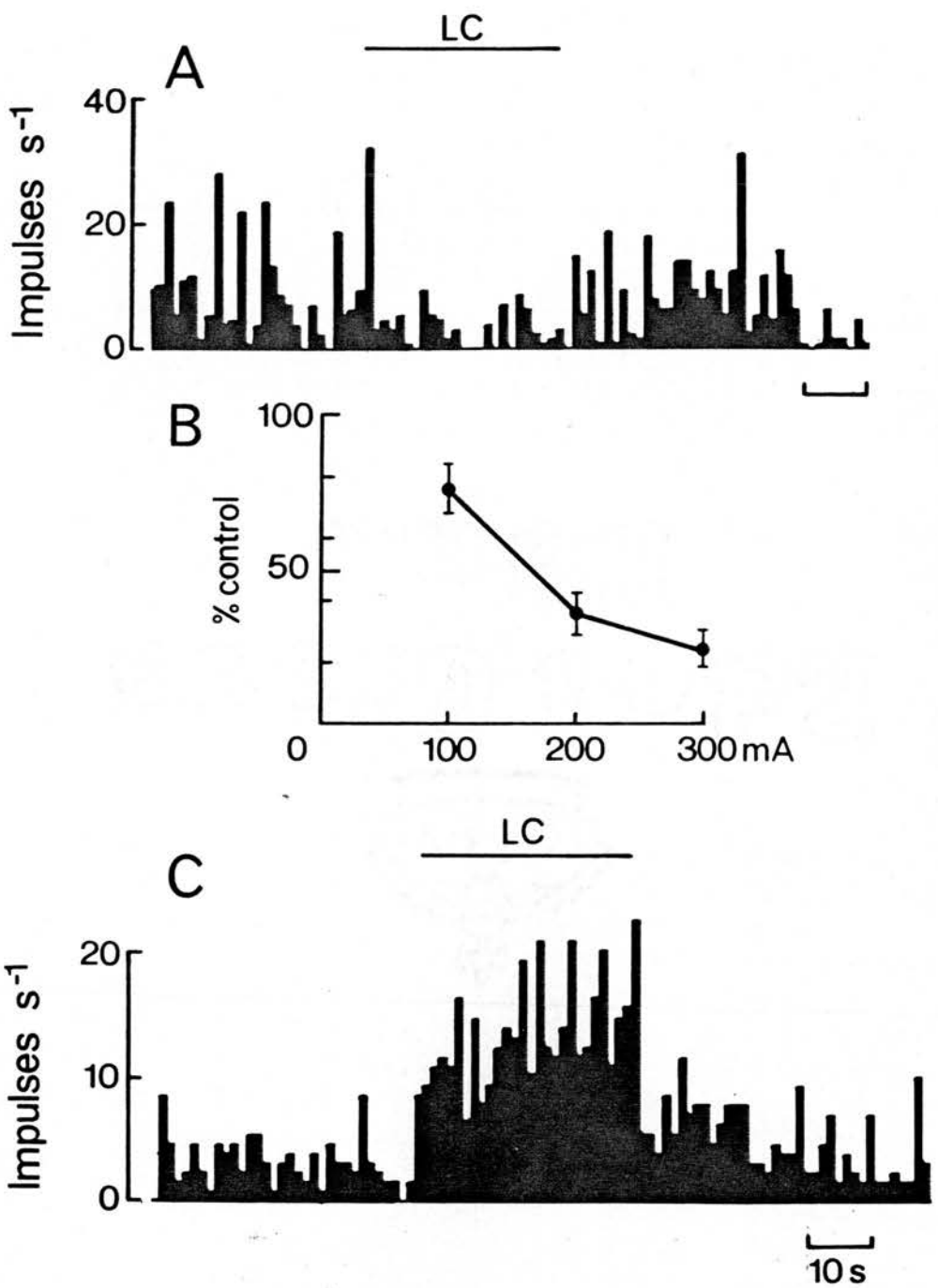

LC
A
40
20
0
Impulses s-1
B
100
50
% control
0
100
200
300 mA
LC
C
20
10
0
Impulses s-1
10 s

FIGURE 4.3

Effect of Locus coeruleus stimulation (LC) on the discharge evoked by noxious stimuli

A. The frequency histograms illustrate the response evoked by pinch. A train of stimuli in the LC (80 m sec, 200 HZ) at 100 and 300 μA was repeated once every second. The duration of the stimulation in LC and the application of pinch are shown by the bars above the histograms. The calibration for time is shown in the figure in B. The interstimulus interval between the noxious stimuli was 3 minutes.

B. Frequency histograms of the action potentials illustrate the response evoked by 48°C heat applied for 10 seconds on another neurone. The inhibitory effect of LC and NRM is shown on this evoked discharge. The duration of application of heat and the stimulation of LC and NRM is as shown by the bars on top of the histograms. The following stimulation parameters were used.

LC	NRM
300 μA	300 μA
80 m sec train	30 m sec train
200 HZ	200 HZ
200 μ sec p. width	200 μ sec p. width
repetition rate 1/sec	repetition rate 1/sec

The thresholds from NRM was >100 μA whereas from LC it was >150 when a 30 m sec train at 200 HZ was repeated once every second.

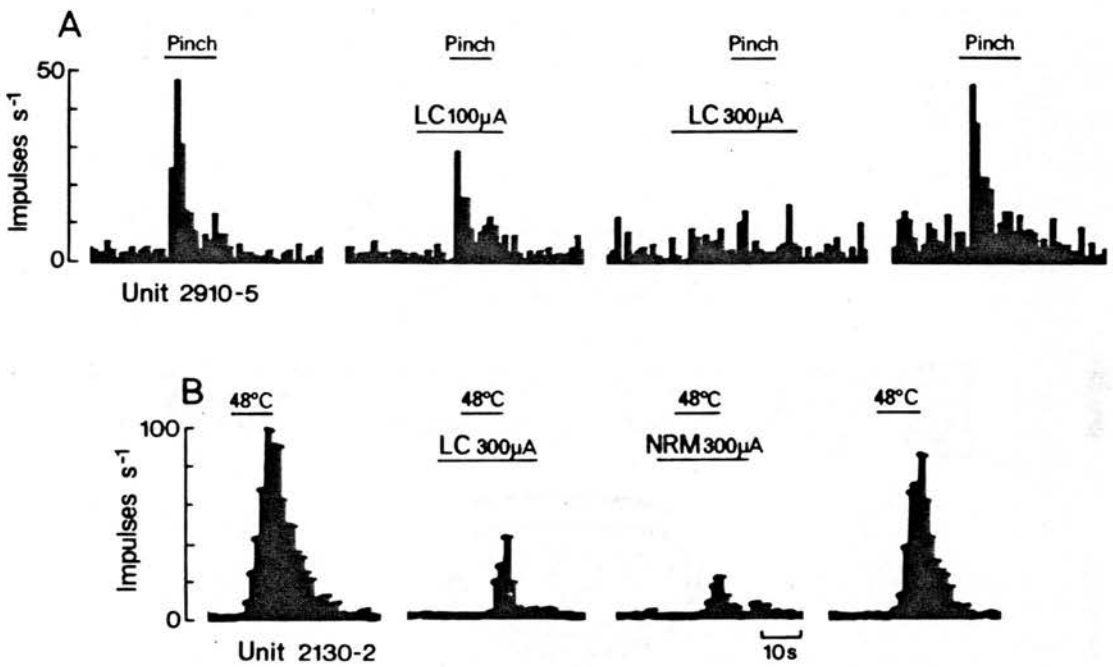

A
Pinch
Pinch
Pinch
Pinch
50
Impulses s-1
0
LC 100μA
LC 300μA
Unit 2910-5
B
48°C
48°C
48°C
48°C
100
Impulses s-1
0
LC 300μA
NRM 300μA
Unit 2130-2
10s

FIGURE 4.4

Effect of stimulation in LC on the discharge evoked by noxious heat of a multireceptive neurone

The figure shows the effect of locus coeruleus stimulation (30 m sec train at 200 HZ) at 300 μA producing an inhibition of discharge evoked by noxious heat pulses (10 sec. duration) at different intensities (48-52°C). The top records show the control response to the heat stimuli at different intensities and the bottom records show the effect of LC stimulation on the discharge evoked by the identical heat stimuli as shown in the control recods. The thin bars represent the application and duration of heat stimulus and the thick bars represent the duration of the LC stimulation.

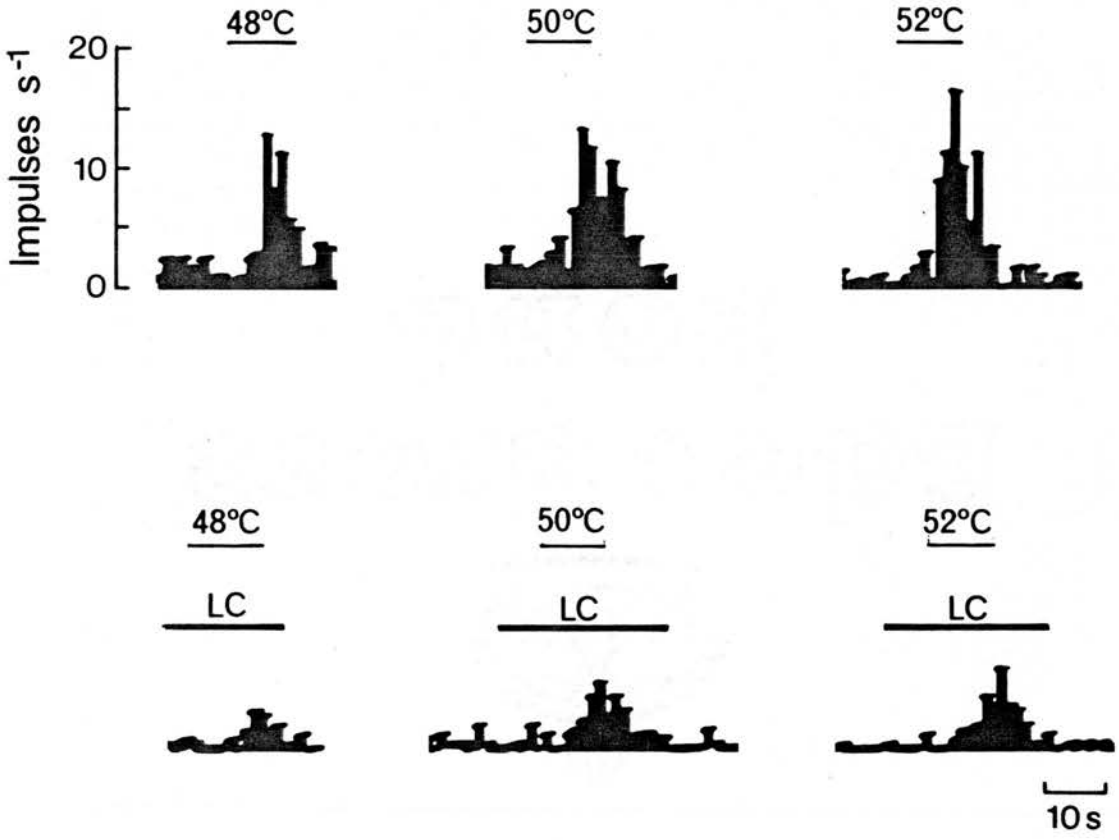

48°C
50°C
52°C
Impulses s-1
20
10
0
48°C
50°C
52°C
LC
LC
LC
10 s

FIGURE 4.5

Effect of stimulation in the nucleus locus coeruleus (LC) on the discharge evoked by increasing skin temperatures (noxious heat stimuli)

This graph shows the control response (open circles) to noxious heat stimuli at different intensities applied for 10 seconds and the response evoked by the identical stimuli in the presence of stimulation in LC (filled circles). Each point represents the spikes counted for a period of 10 seconds. This multireceptive neurone did not have any background discharge.

The LC stimulation parameters were as follows:

300 μA
80 m sec train
200 HZ
200 μsec pulse width
repetition rate 1/sec

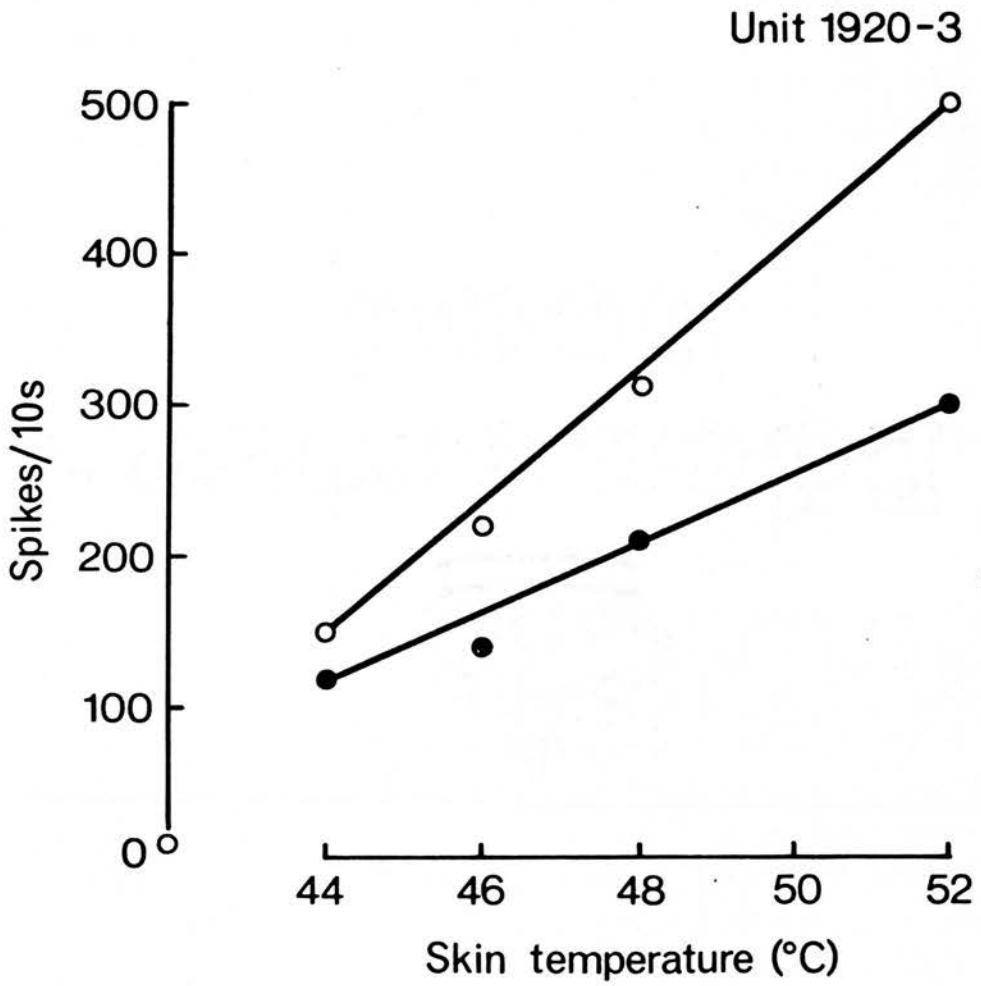

Unit 1920-3
500
400
300
200
100
0
Spikes/10s
44
46
48
50
52
Skin temperature (°C)

FIGURE 4.6

Time course of the inhibition from LC produced on the evoked responses to heat

The graph shows the effect produced on the evoked response to heat on applying the noxious heat stimulus (48°C for 10 s) at various times during the application of the locus coeruleus stimulation (40 seconds). Each point represents the spikes counted for a period of 10 seconds starting 5 seconds after the onset of the heat stimulus and is plotted at the time of starting the count each time. The inhibitory effect of LC is first observed as shown when the heat was applied five seconds before the onset of LC stimulation. Strong inhibition of the response evoked by heat was seen on applying the heat stimulus at the onset of LC stimulation and a similar level of inhibition is observed when heat was applied at various times after the onset of LC. There was no inhibition observed when the heat stimulus was applied 5 seconds before LC was turned off (at 35 seconds after the onset of LC) which is plotted here at 40 seconds which is the start of the counting period.

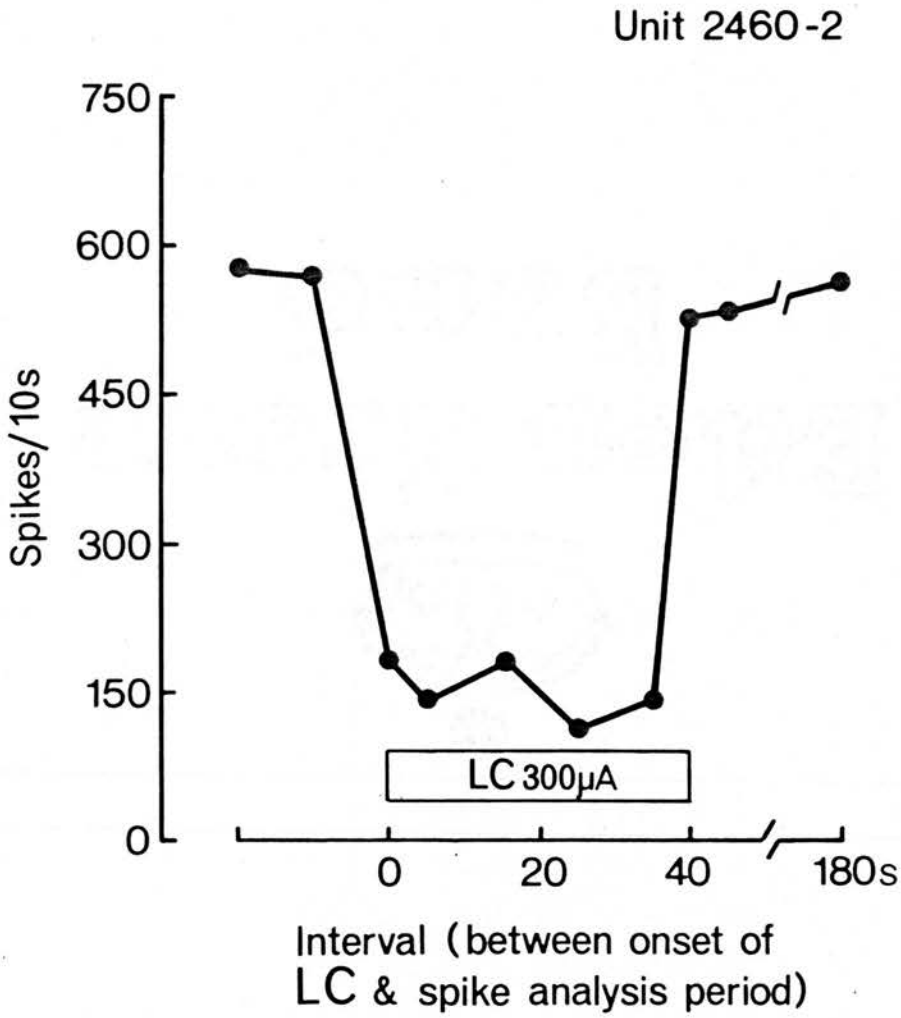
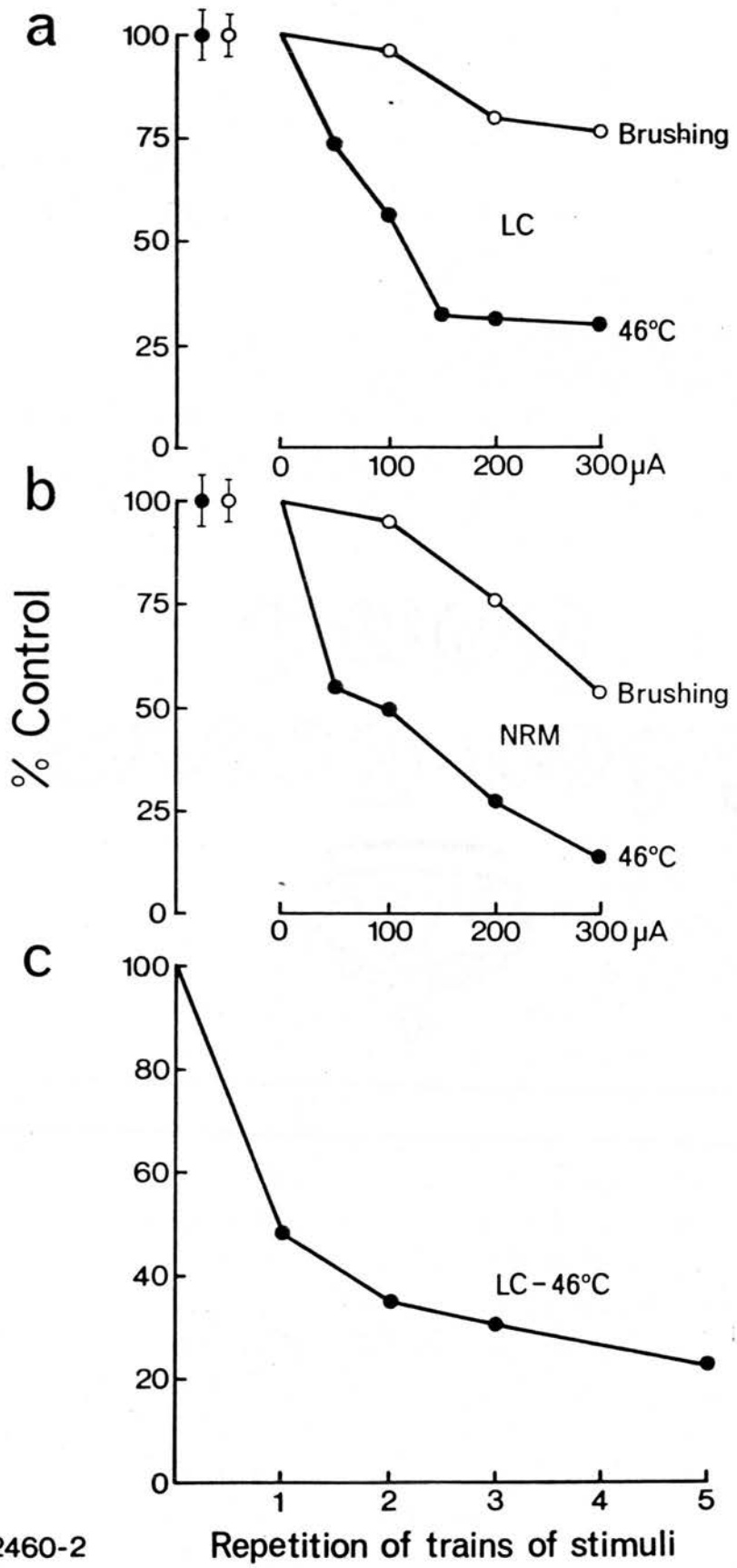

Unit 2460-2
750
600
450
300
150
0
Spikes/10s
LC 300μA
0
20
40
180s
Interval (between onset of
LC & spike analysis period)

FIGURE 4.7

Effect of stimulation in the locus coeruleus (LC) and raphe magnus (NRM) on the response evoked by noxious and non-noxious cutaneous stimulation

a,b The graphs show the effect of LC and NRM stimulation at increasing the current intensities on the response evoked by noxious heat (filled black circles) and on the discharge produced by the non-noxious brushing of the receptive field (open circles). The effect of LC is shown as a percentage of the control response (without LC stimulation). The variability in the control response to heat and brushing is shown at the top left hand corner of each graph. The heat stimulus at 46°C was applied for a period of 10 seconds and the interstimulus interval was 3 minutes. Both LC and NRM were stimulated with trains of 30 m sec at 200 HZ repeated twice a second.

c The graph shows the effect of increasing the repetition rate of a train of stimuli (30 m sec at 200 HZ) at a current intensity of 300 μA in the LC on the evoked discharge to heat represented by the filled black circles. The numbers on the abcissa represent the frequency of stimulation (HZ).


a
100
75
50
25
0
Brushing
LC
46°C
0
100
200
300µA
b
% Control
100
75
50
25
0
Brushing
NRM
46°C
0
100
200
300µA
c
100
80
60
40
20
0
LC-46°C
1
2
3
4
5
Repetition of trains of stimuli

2460-2

FIGURE 4.8

The Dot-raster display for this and subsequent figures

The figures show the effect of LC and NRM stimulation on the spontaneous and evoked discharge of four different neurones (a, b, c, d) using the dot raster display technique. The dot rasters shown in this figure and in the figures that follow read from left to right and down like reading a book. The duration of the application of the conditioning and test stimuli are shown by the bars on the left-hand side of each dot-raster. Each dot represents one actional potential. Each horizontal sweep was triggered by the stimulator (triggering pulse) and successive responses are displayed below the preceding one. In this and subsequent figures if there was an interval between the conditioning (LC or NRM) and the test stimulus (electrical or cutaneous) it is indicated by arrows at the top. The calibration for the sweep speed (time) is shown on the right-hand corner of each figure. The LC stimulation is represented by a black bar and the NRM stimulation by a stippled bar whereas the electrical or cutaneous stimuli are shown by open bars in this and subsequent figures.

FIGURE 4.8

a : Effect of stimulation in the LC and NRM on the discharge evoked by the tibial nerve stimulation at a conditioning test interval of 40 ms. The first few traces show the background discharge. Then a test stimulus (20V, 1 ms, 1.2 sec interval) was applied to the tibial nerve 40 m sec after the start of each sweep. After recording several responses to the test stimulus (T) the locus coeruleus was stimulated with 80 m sec trains (300 μA, 200 μ sec, 330 HZ) at the start of each trace. This whole sequence was then repeated while stimulating NRM at the same parameters as for LC. Stimulation of LC and NRM inhibited the response evoked by the tibial nerve stimulation the inhibition from LC was stronger than from NRM.

b : This record taken from another unit shows an equal magnitude of inhibition produced from LC and NRM on the discharge evoked by pinching the receptive field of the unit. The first few traces show the background discharge followed by the response to pinch. Stimulation of LC and NRM (300 μA, 200 μ sec pulses, 300 HZ, 80 m sec train, repetition rate 1 HZ) inhibited this evoked discharge as shown in the dot raster.

c : The dot-raster shows the effects of stimulation in the LC and NRM on the discharge evoked by the tibial nerve (TN) stimulation (2V, 0.5 m sec pulse width) at a conditioning (C) test (T) interval of 40 m sec. Stimulation in LC (300 μA, 60 m sec train, repeated every 1.2 second) produced an early excitation followed by a period of inhibition of the evoked discharge. Similar parameters of stimulation in NRM evoked inhibition only.

d : This record shows the effect of stimulation in LC and NRM on the background discharge of another multireceptive neurone. Stimulation of LC and NRM (300 μA, 200 μ sec pulse width, 100 m sec train length, 200 HZ, rep. rate 2.2 sec) produced inhibition lasting longer than a second. NRM produced an early period of strong excitation as well. The numbers underneath each figure at the bottom left-hand corner show the unit number under investigation.

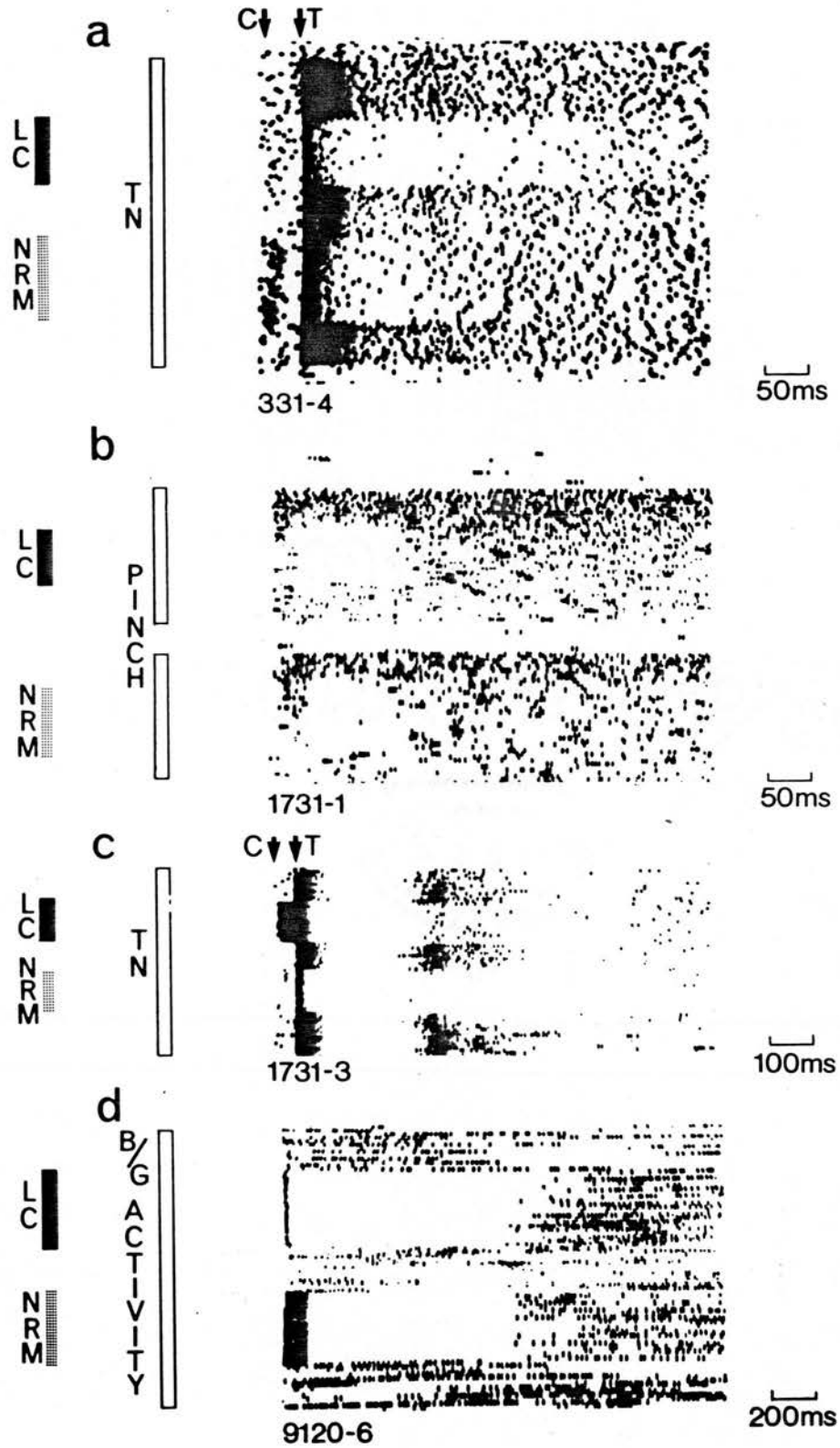

a
C
T
LC
NRM
TN
331-4
50ms
b
LC
NRM
PINCH
1731-1
50ms
c
C
T
LC
NRM
TN
1731-3
100ms
d
LC
NRM
B/G ACTIVITY
9120-6
200ms

FIGURE 4.9

Effect of stimulation in locus coeruleus on the discharge of a mechanoreceptive neurone (low threshold or Class 1)

The discharge of this mechanoreceptive neurone is shown in the frequency histograms illustrating the response to brushing the receptive field as indicated by thin bars in the absence (control) and in the presence of stimulation in the locus coeruleus (represented by upper thick bars in the middle record). The parameters of stimulation in LC were as follows:

Current intensity	-	300 μA
Pulse width	-	200 μ sec
Train length	-	80 m sec
Interpulse interval	-	2 m sec (500 HZ)
Repetition rate	-	1-5/sec

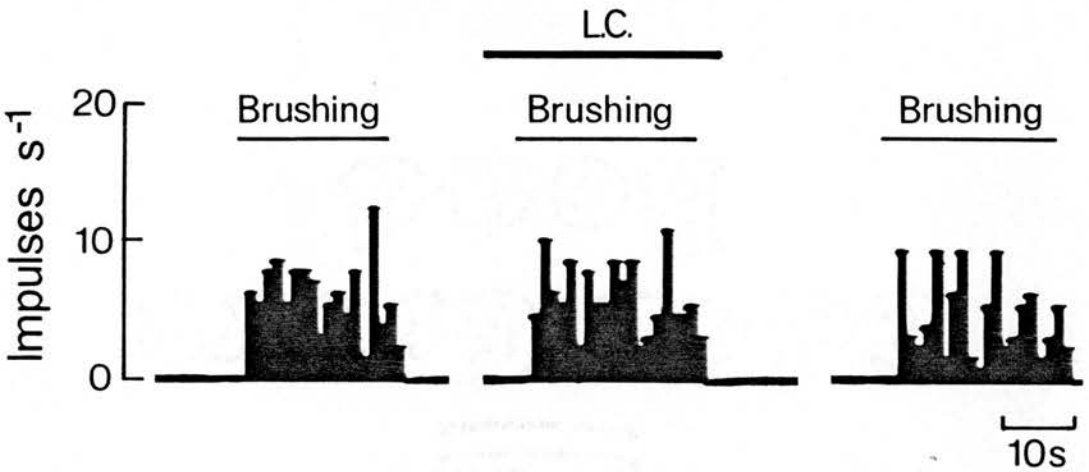

L.C.
Brushing
Brushing
Brushing
Impulses s-1
20
10
0
10s

FIGURE 4.10

Location of recording sites in the spinal cord

a,b : The figures show composite pictures of the majority of the recording sites which were marked by the electrophoretic deposition of the pontamine dye in the lumbar spinal cord. The keys for these figures are shown at the bottom on the right-hand corner of each figure. "LC test" in the figure (a) means that only the effect of nucleus locus coeruleus stimulation was studied whereas "LC and NRM test" (a, b) means that the effect of stimulating both the nuclei was studied on a particular neurone represented by the filled triangles. The unfilled squares show locations of mechano-receptive neurones (Class 1) whereas all the remaining units are of the multireceptive type (responding both to noxious and non-noxious cutaneous stimulation).

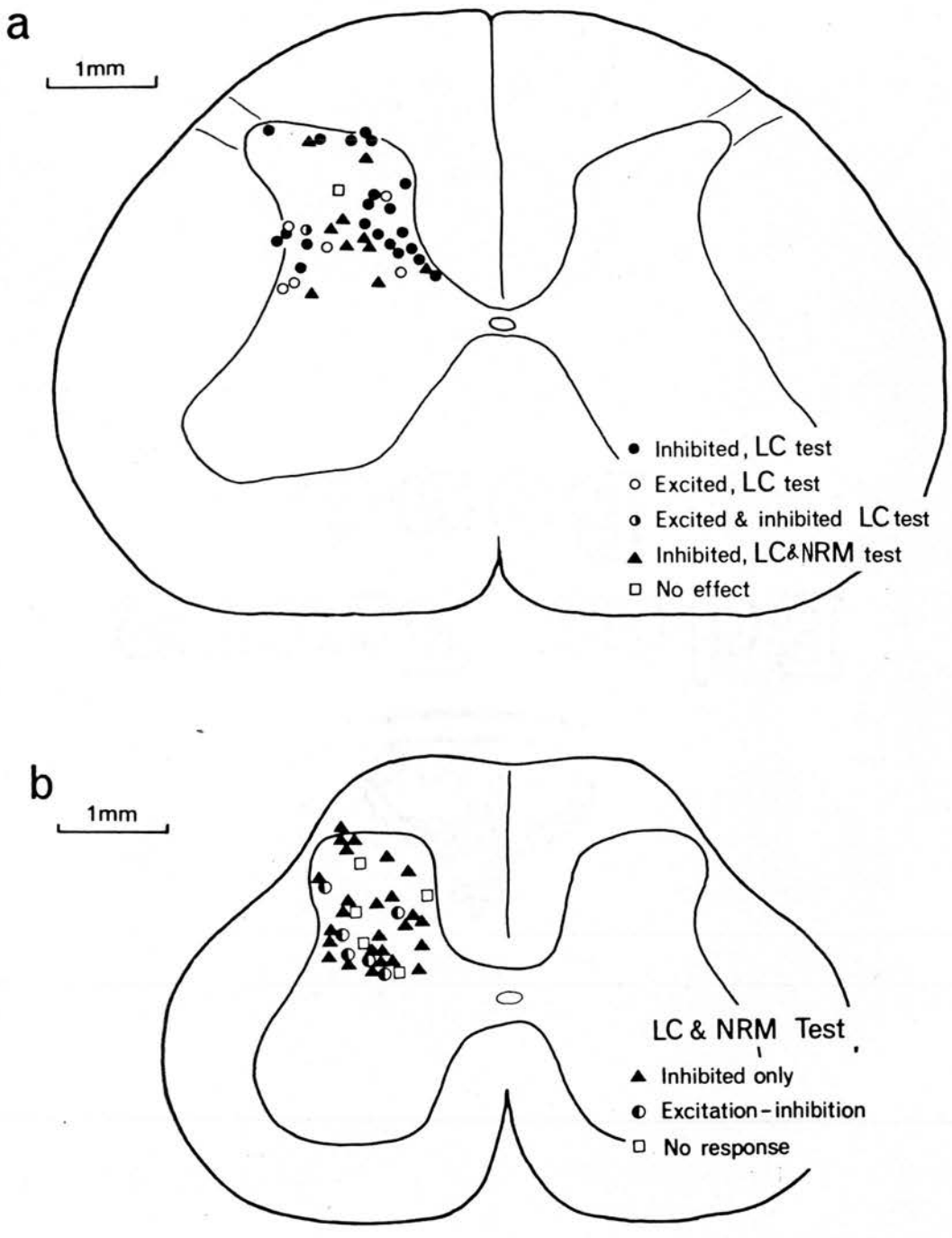

a
1mm
Inhibited, LC test
Excited, LC test
Excited & inhibited LC test
Inhibited, LC&NRM test
No effect
b
1mm
LC & NRM Test
Inhibited only
Excitation-inhibition
No response

FIGURE 4.11

(P2 - P4)

Abbreviations:

5M	:	motor trigeminal nucleus
5ME	:	mesencephalic trigeminal nucleus
5N	:	trigeminal nerve
BC	:	brachium conjunctivum
BP	:	brachium pontis
BCM	:	marginal nucleus of the brachium conjunctivum
CS	:	superior central nucleus
DRM	:	dorsal nucleus of the raphe, medial division
IC	:	inferior colliculus
LC	:	nucleus locus coeruleus
MLB	:	medial longitudinal bundle
P	:	pyramidal tract
SC	:	sub-coeruleus
SOM	:	medial nucleus of the superior olive
TB	:	trapezoid body
TDP	:	dorsal tegmental nucleus pericentral division
V4	:	fourth ventricle

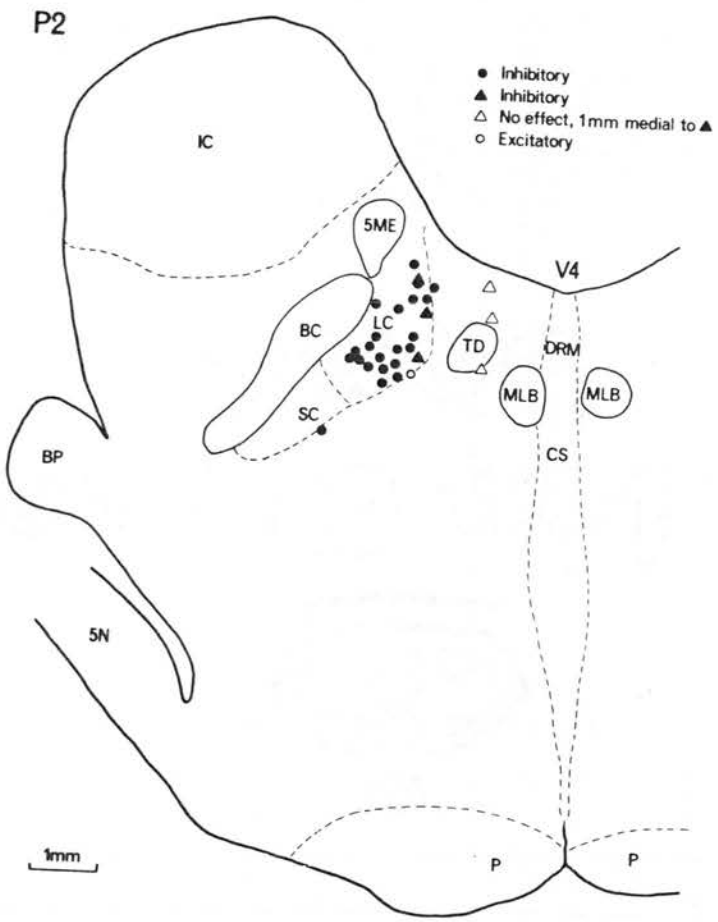
TD : dorsal tegmental nucleus

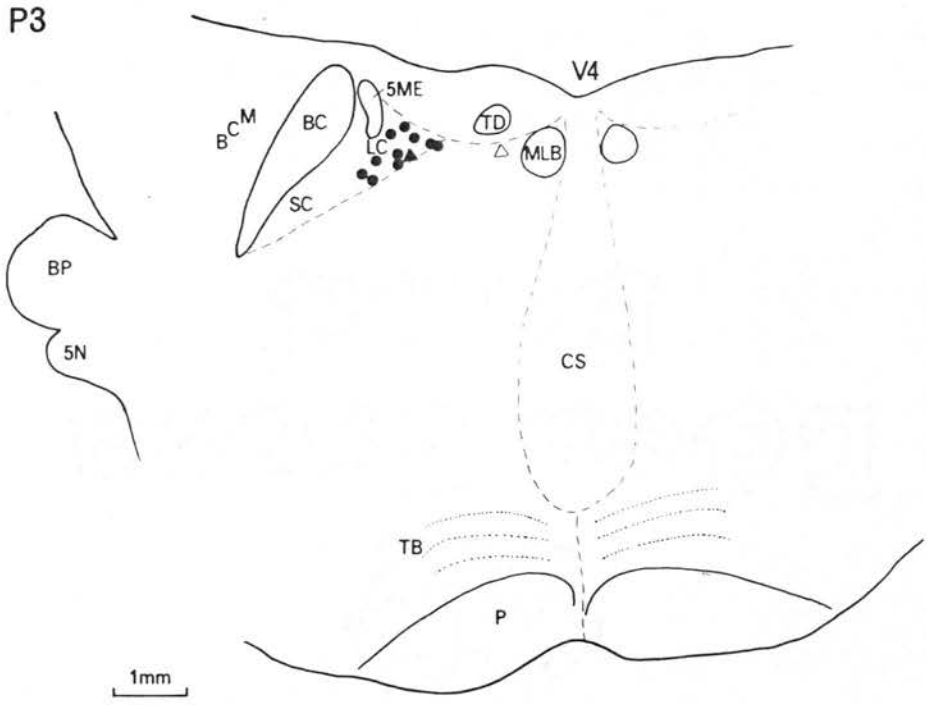
FIGURE 4.11

(P2 - P4)

Locations of the LC stimulation sites

The figures from P2-P4 are composite pictures showing the sites of stimulation at the posterior co-ordinates P2-4. Filled circles represent the sites of stimulation producing inhibition whereas the open circles represent sites producing excitation on the dorsal horn neurones. The filled triangles at P2 and P3 represent the sites of stimulation which were effective in producing inhibition and the open triangles represent sites which were situated 1 mm medial to the effective site of stimulation (filled triangles) but did not produce any effect on using similar parameters of stimulation which evoked inhibition from the effective sites shown by filled triangles. The open and the filled triangles at each place correspond to the position of the medial and the lateral electrode in the pair. Many ineffective sites corresponding to the position of the medial electrode in the pair have not been shown. The lateral electrode lying within the nucleus locus coeruleus was always the most effective site producing inhibition, or excitation and inhibition.


P2
● Inhibitory
▲ Inhibitory
△ No effect, 1mm medial to ▲
○ Excitatory
IC
5ME
V4
BC
LC
TD
DRM
MLB
MLB
SC
BP
CS
5N
1mm
P
P


P3
V4
5ME
BC
M
BC
LC
TD
MLB
SC
BP
5N
CS
TB
P
1mm

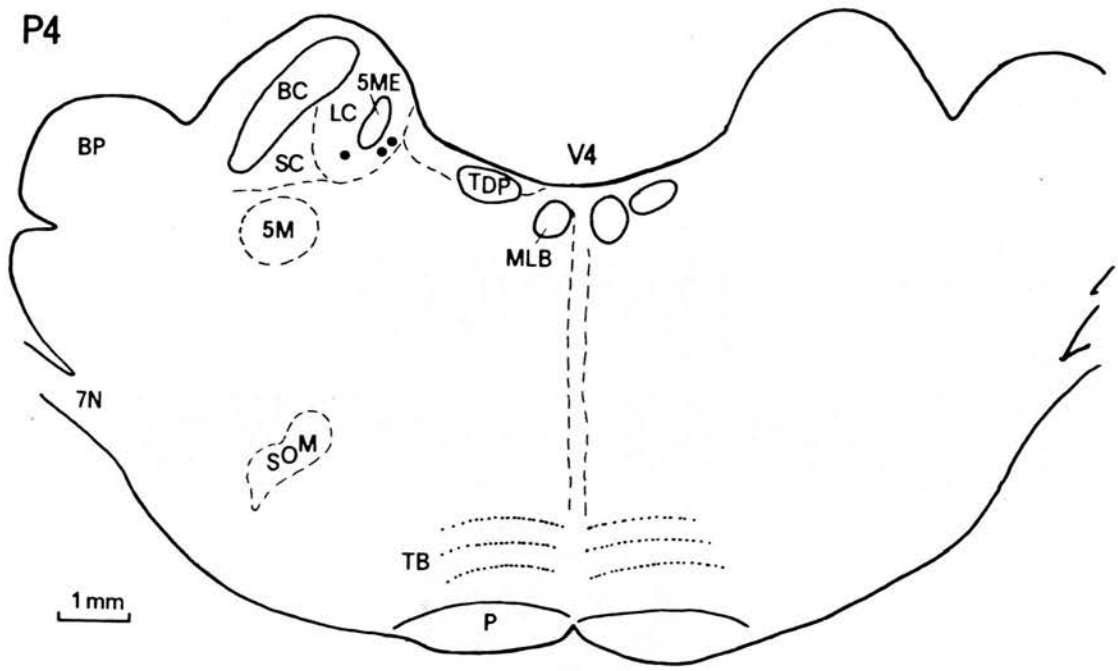

P4
BC
5ME
LC
BP
SC
V4
TDP
5M
MLB
7N
SOM
TB
1 mm
P

FIGURE 4.12

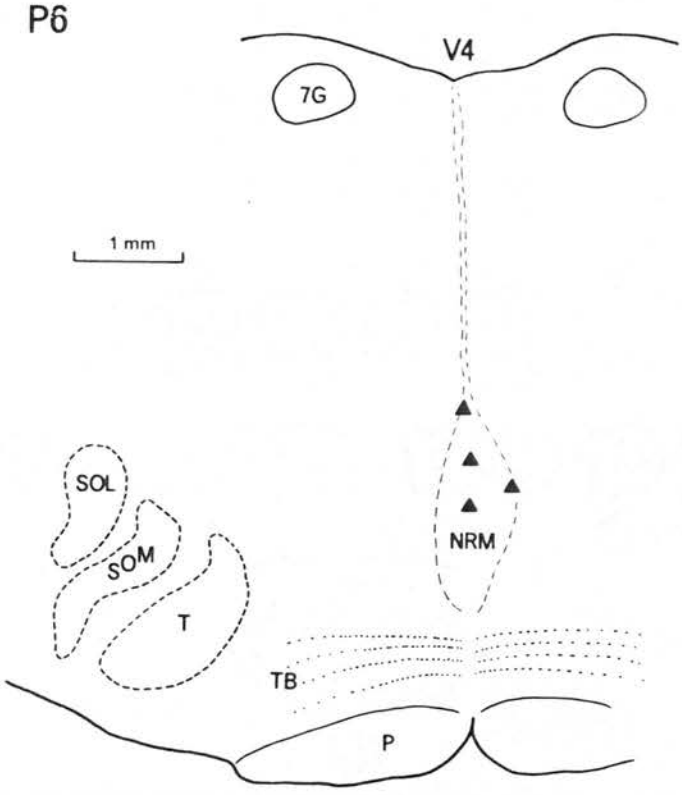
(P6 - P8)

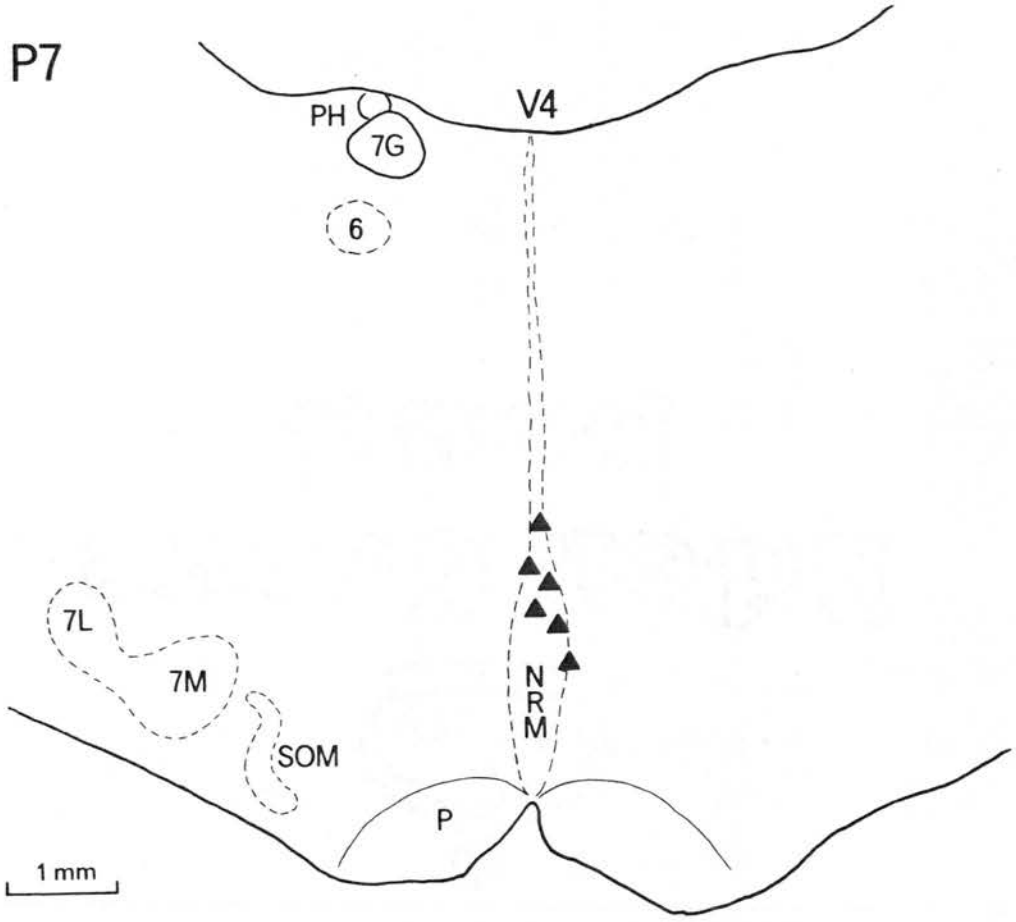
Locations of the sites of stimulation in the NRM

These are composite pictures showing the sites of stimulation in or adjacent to the NRM at the posterior co-cordinates P6-8. The filled triangles represent the sites of stimulation that produced inhibition, or excitation and inhibition of the discharge of multireceptive neurones.

Abbreviations:

6	:	abducens nucleus
7G	:	genu of the facial nerve
7L	:	facial nucleus, lateral division
7M	:	facial nucleus, medial division
NRM	:	nucleus raphe magnus
P	:	pyramidal tract
PH	:	nucleus praepositus hypoglossi
PPR	:	postpyramidal nucleus of the raphe
SOL,M	:	lateral, medial nucleus of the superior olive
T	:	nucleus of the trapezoid body
TB	:	trapezoid body
V4	:	fourth ventricle


P6
V4
7G
1 mm
SOL
SOM
T
NRM
TB
P


P7
PH
7G
V4
6
7L
7M
SOM
NRM
P
1 mm

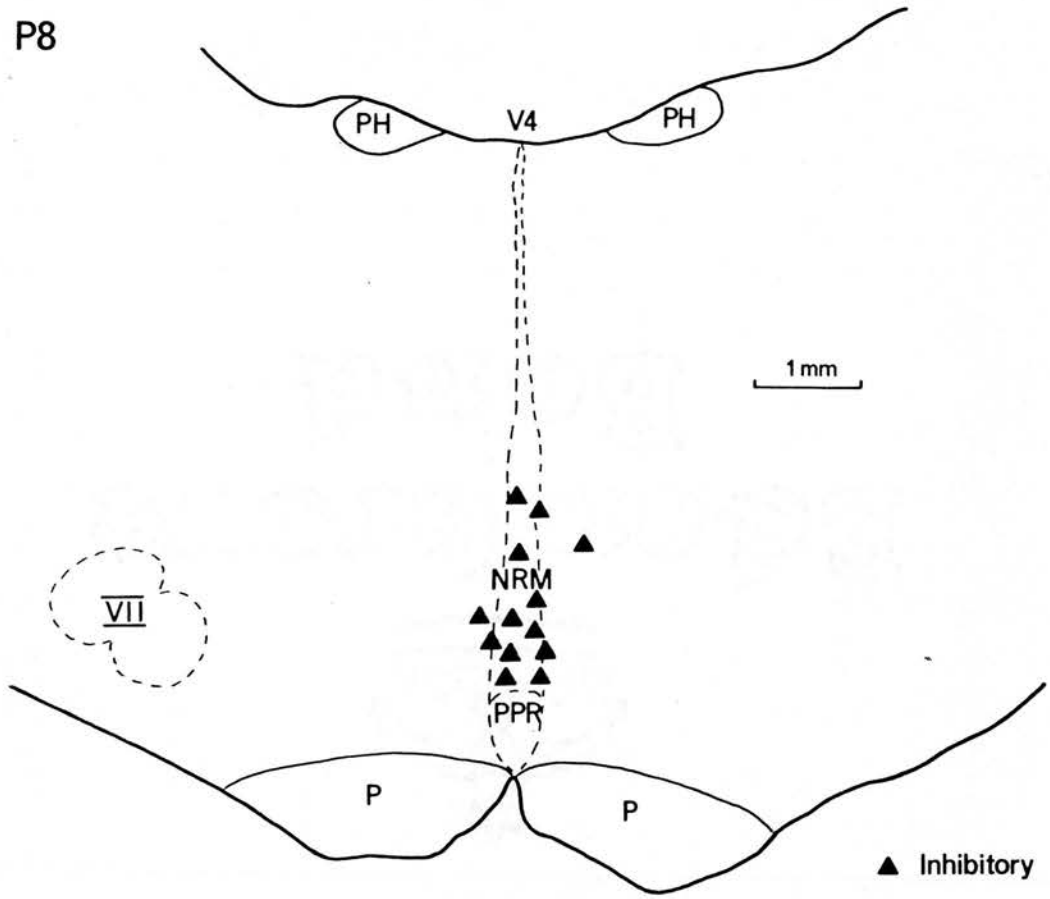

P8
PH
V4
PH
1 mm
NRM
VII
PPR
P
P
Inhibitory

FIGURE 4.13

The effect of phenoxybenzamine (PBZ) on the inhibition produced by the stimulation of LC on the spontaneous activity (SA) and the heat evoked discharge of a multireceptive neurone

The graph shows the effect of phenoxybenzamine (PBZ) administered intravenously on the inhibition of this unit produced by stimulation in LC on the response evoked by heat (filled triangles) and on the spontaneous activity (filled circles). The response evoked by heat and the spontaneous activity are shown in the presence of LC stimulation as a percentage of the control response prior to and after the drug administration. The reduction in the LC inhibition produced by phenoxybenzamine builds over a period of time (see text). The variability in the control response evoked by heat and the spontaneous activity (filled circles) is shown at the top left-hand corner of the graph. A 80 m sec train (500 HZ) at 300 μA was repeated once every second to produce inhibition from LC at the times plotted in the graph. The threshold for producing the inhibition was 100 μA.

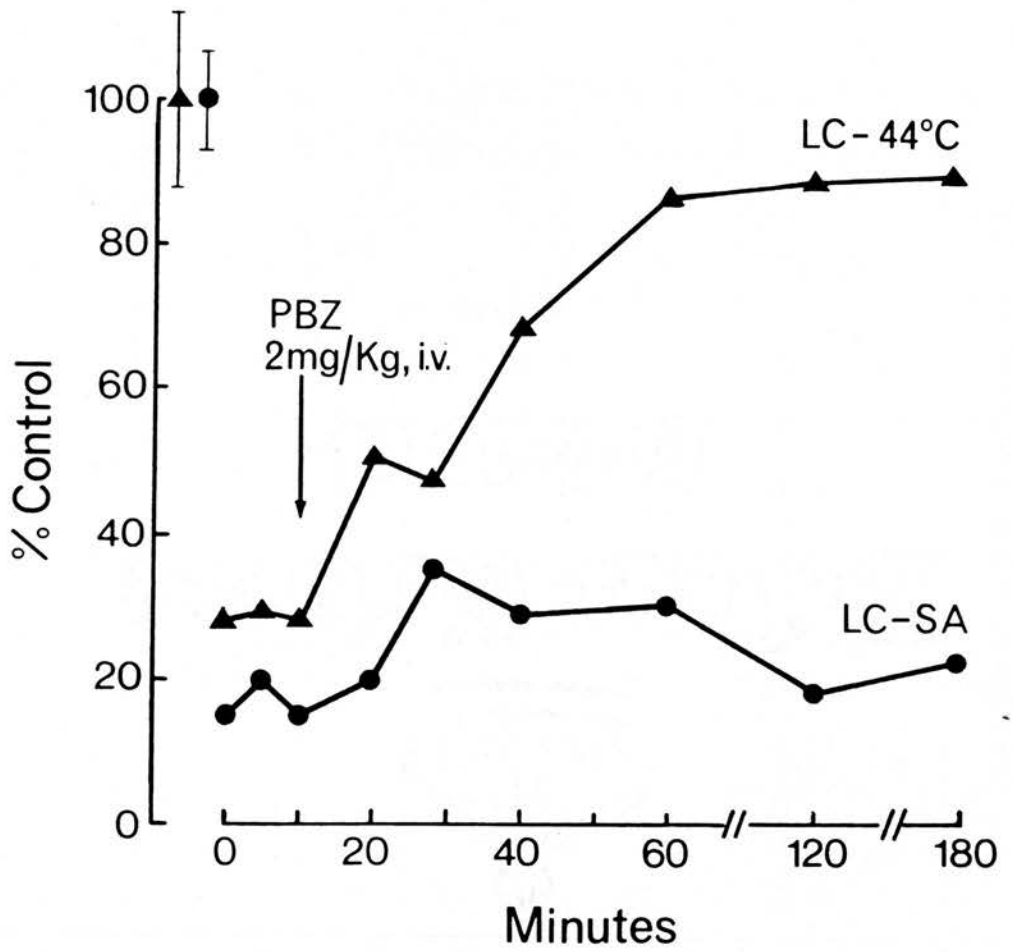

100
80
60
40
20
0
% Control
PBZ
2mg/Kg, i.v.
LC - 44°C
LC-SA
0
20
40
60
120
180
Minutes

FIGURE 4.14

Effect of yohimbine on the inhibition produced by LC stimulation on the evoked response to heat

The thin bars illustrate the response of this unit to pinch and the lower thick bars show the inhibition of these responses during stimulation of the nucleus locus coeruleus. There is an almost complete inhibition of the response to pinch by stimulation in the LC prior to the administration of yohimbine. After the intravenous administration of yohimbine (2 mgm/kg) (arrow) the inhibition produced from LC on the evoked response to pinch was strongly antagonised. The interstimulus interval of 5 minutes between the noxious stimuli was kept constant. The stimulation parameters of LC stimulation were kept constant throughout (300 μA, 80 m sec, 500 HZ, 200 μ sec pulse width, repetition rate 1/sec).

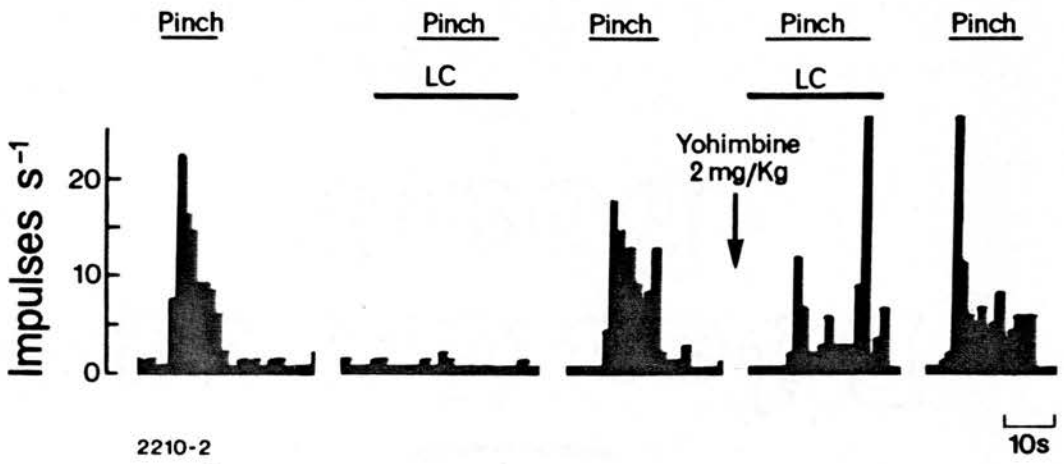

Pinch
Pinch
Pinch
Pinch
Pinch
LC
LC
Yohimbine
2 mg/Kg
Impulses s-1
20
10
0
2210-2
10s

FIGURE 4.15

The effect of propranolol and sotalol on the inhibition produced from LC on evoked discharge and the spontaneous discharge

a : The frequency histograms illustrating the evoked response to light pressure (LPr) and pinch applied to the receptive* of a multireceptive neurone. The thin bars show the duration of application of the cutaneous stimulation (LPr or pinch) and thick bars represent the duration of stimulation of locus coeruleus (LC). A train of 80 m sec (500 HZ) at 300 μA in LC produced a similar magnitude of inhibition on the response evoked by light pressure and pinch.

b : The frequency histograms illustrating the evoked response to light pressure and pinch of the same neurone as shown above (a) but after the administration of propranolol (1 mgm/kg i.v.) (arrow). The response to cutaneous stimulation increased as shown but the inhibitory effect of the stimulation of locus coeruleus was still intact.

c : These frequency histograms show background activity of another neurone and the inhibition produced by LC stimulation prior to and after the administration of sotalol (arrow). The spontaneous activity increased but the inhibition from LC was not antagonised by sotalol.

*field

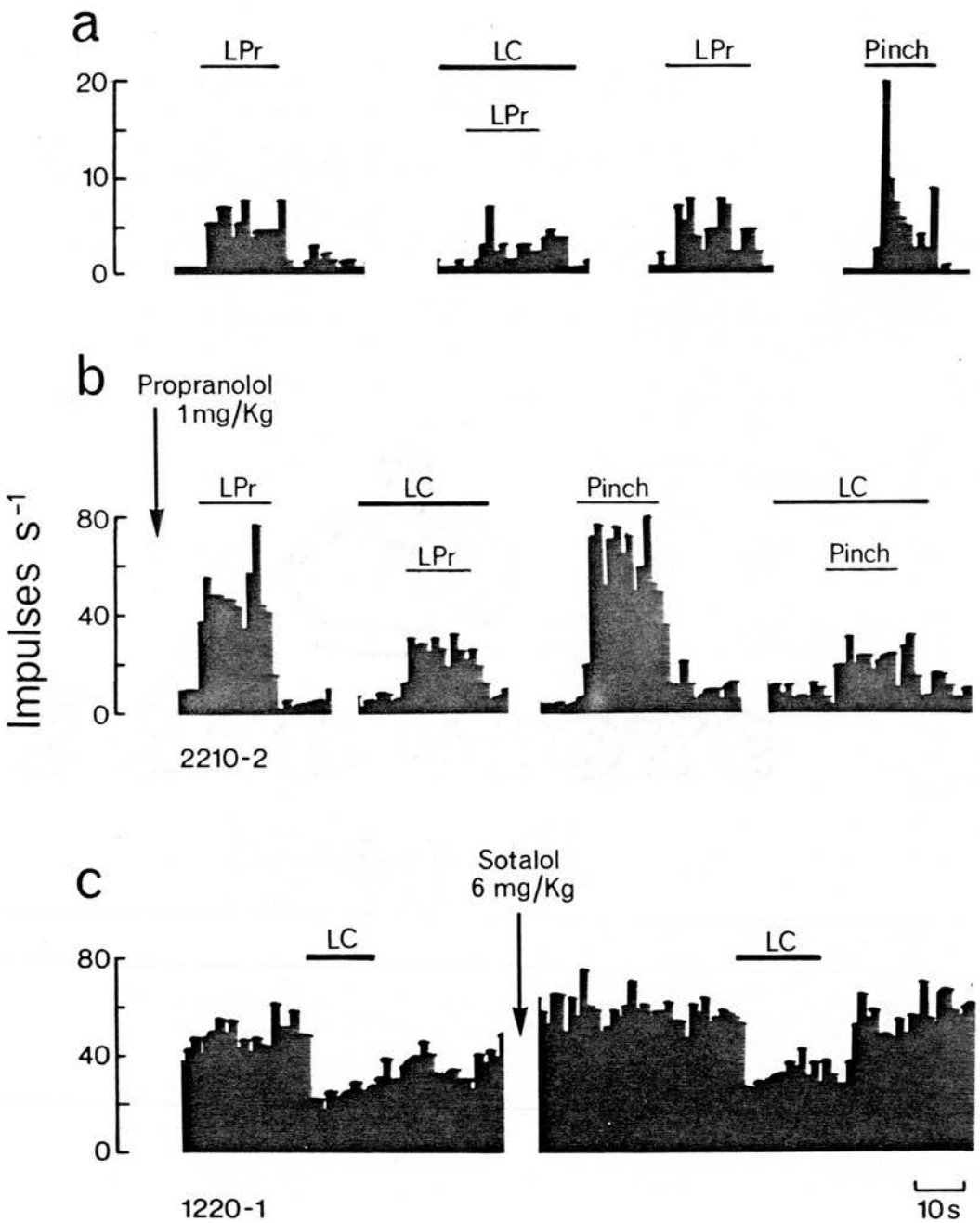

a
LPr
LC
LPr
Pinch
LPr
20
10
0
b
Propranolol
1mg/Kg
LPr
LC
Pinch
LC
LPr
Pinch
80
40
0
Impulses s-1
2210-2
c
Sotalol
6 mg/Kg
LC
LC
80
40
0
1220-1
10s

FIGURE 4.16

The effect of methysergide on the actions produced by the stimulation of LC and NRM

a : Before the administration of methysergide
The raster display of the activity evoked by the electrical stimulation of the tibial nerve (2 V) repeated after every 1.2 seconds represented by the unfilled bar. Stimulation of LC (filled black bar) and NRM (stippled bar) produced an inhibition of the evoked discharge at a conditioning (C) test (T) interval of 80 m sec. Stimulation of NRM also produced an early excitation, as shown. The application of the conditioning (C) test (T) stimuli are shown by arrows on top of the raster display. A train of 60 m sec (200 HZ) at 300 μA was used to stimulate the LC and NRM.

b : After the administration of methysergide
The raster display of the same unit after administering methysergide (6 mgm/kg). The excitation from NRM was abolished as shown but the inhibition from LC and NRM was not antagonised. The spontaneous activity shown in figure (a) above was also abolished. All the stimulation parameters were the same as above (a).

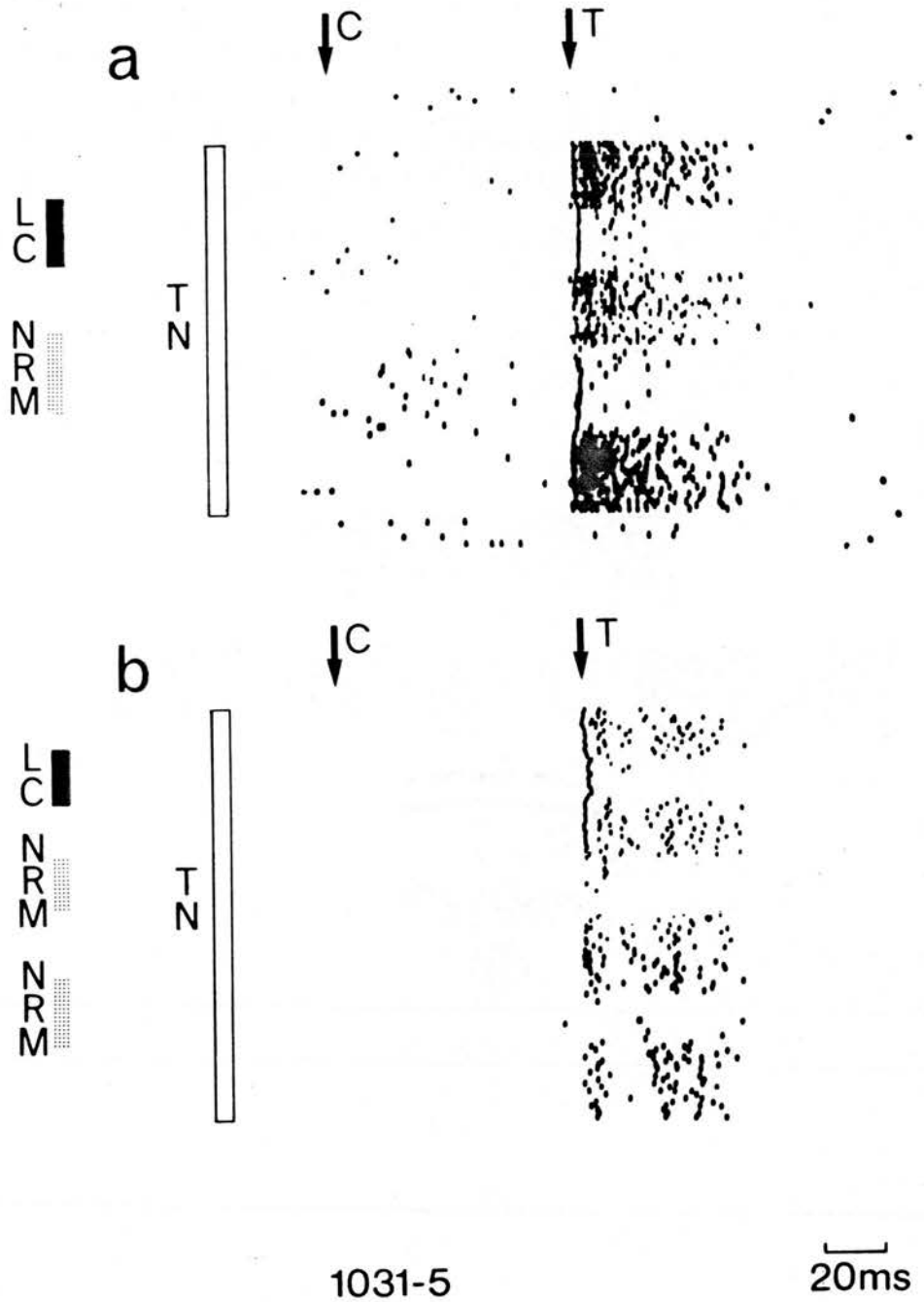

a
C
T
LC
NRM
TN
b
C
T
LC
NRM
NRM
TN
1031-5
20ms

FIGURE 4.17

The effect of methysergide on the inhibition produced by stimulation of LC and NRM on the response evoked by heat

The frequency histograms show the response evoked by heat (46°C) in the presence and absence of stimulation of LC and NRM respectively. The bottom part of the figure shows the control response to heat stimulus applied alone and in the presence of LC and NRM stimulation*after the administration of methysergide (arrow). The heat stimulus and the parameters of electrical stimuls were kept the same throughout.

* immediately

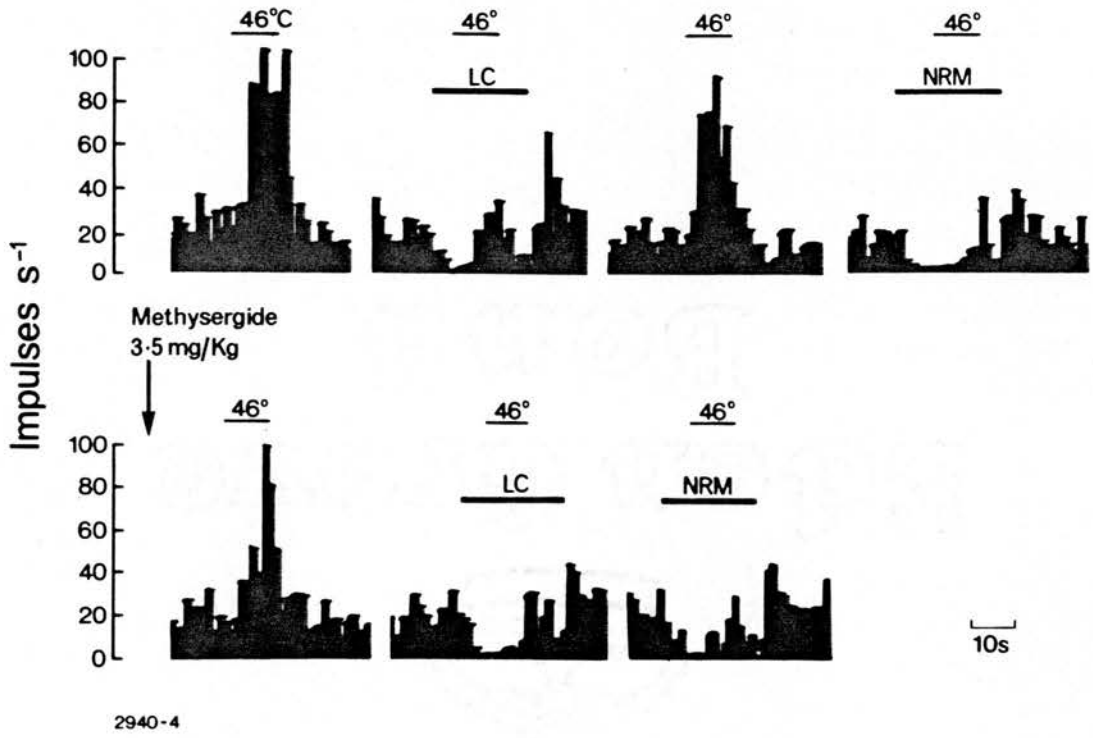

46°C
46°
LC
46°
46°
NRM
Impulses s⁻¹
100
80
60
40
20
0
Methysergide
3·5 mg/Kg
46°
46°
LC
46°
NRM
10s
2940-4

FIGURE 4.18

The effect of naloxone on the inhibition evoked from LC and NRM on the discharge produced by the electrical stimulation

a : Before the administration of naloxone

Raster-display of activity evoked by the electrical stimulation of the dorsal root (10 V) prior to the administration of naloxone. The effect of conditioning stimulation (C) (indicated by arrows) applied in the LC and NRM, 100 m sec prior to the test stimulus applied to the dorsal root (DR) is shown.

b : After naloxone administration

Raster-display showing the repetition of the whole sequence as above (a) but 8.5 minutes after the administration of naloxone (1 mgm/kg).

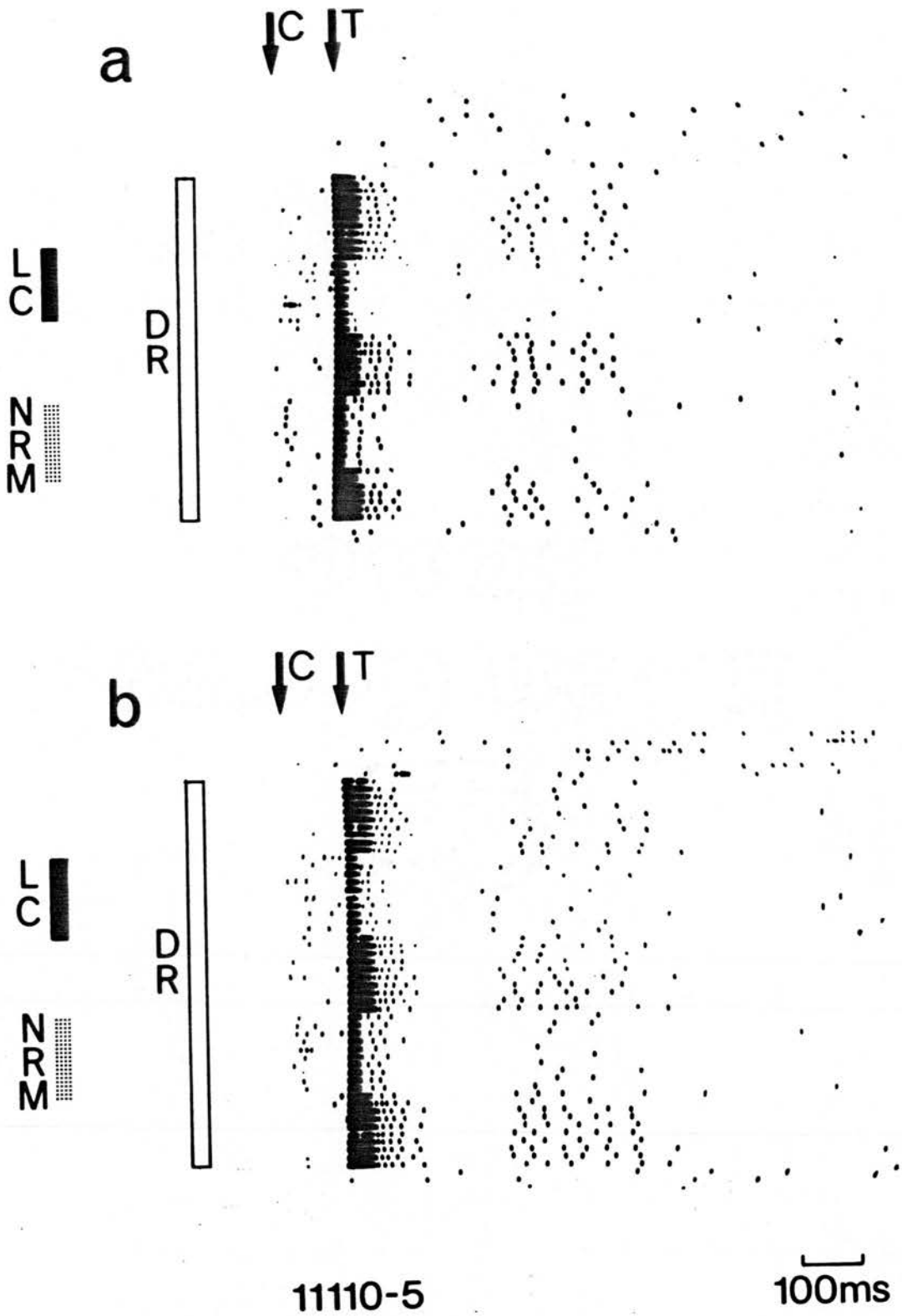

a
C
T
LC
DR
NRM
b
C
T
LC
DR
NRM
11110-5
100ms

FIGURE 4.19

The effect of naloxone on the inhibition of the pinch evoked discharge of a multireceptive neurone produced by stimulation in the locus coeruleus (LC) and the raphe magnus (NRM)

a : Before naloxone

Raster-display showing the response evoked by pinch and the effect of stimulation in the LC and NRM on this response. A train of 60 m sec (200 HZ) at 300 μA was applied in the LC and NRM.

b : After naloxone

Raster-display of the whole sequence as above repeated 1 minute after the administration of naloxone (1 mgm/kg).

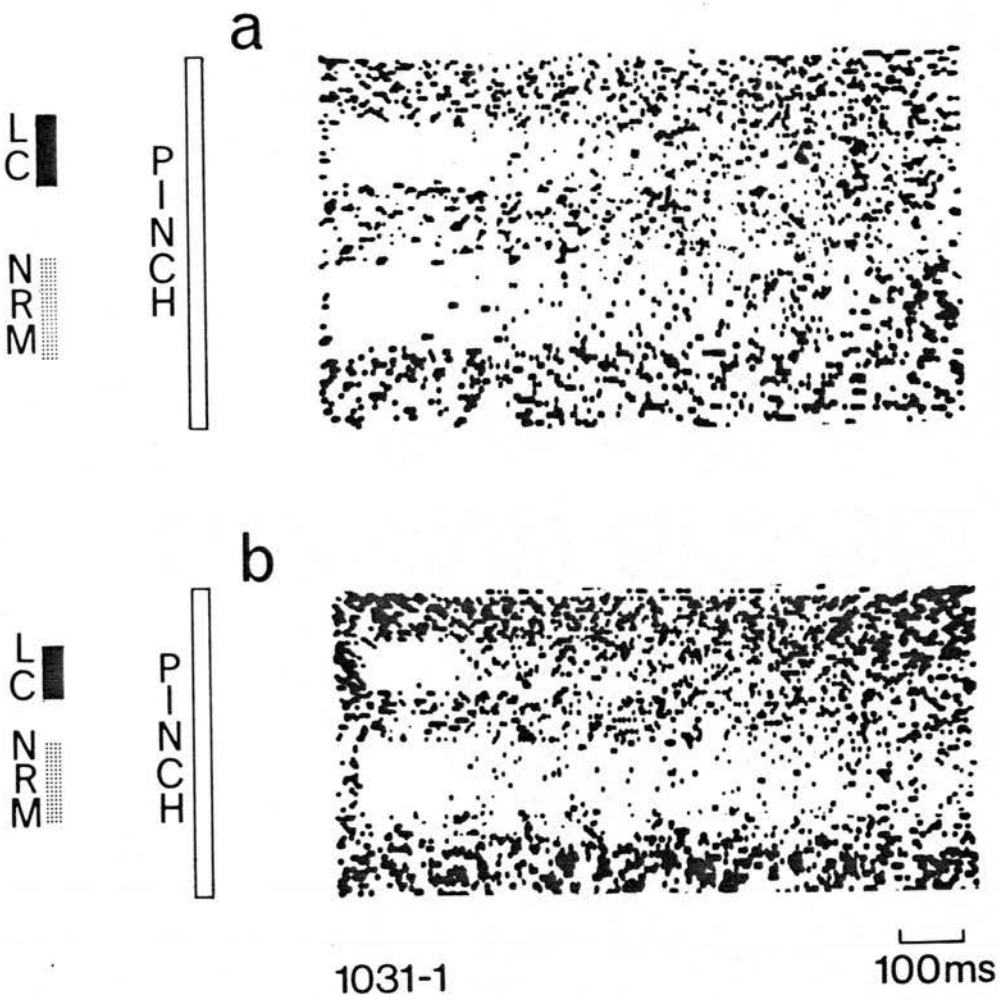

a
LC
NRM
PINCH
b
LC
NRM
PINCH
1031-1
100ms

FIGURE 4.20

The effect of bicuculline on the inhibition produced from LC and NRM

a : Before the administration of bicuculline

The raster display of the discharge evoked by the noxious cutaneous stimulation (pinch) is indicated by the unfilled bar and the effect of stimulation in the LC and NRM on the evoked discharge is shown. A train of 60 m sec (200 HZ) at 300 μA was repeated at 2.2 seconds to stimulate the LC and NRM.

b : After the administration of bicuculline

This raster display shows a similar sequence as above but 3 minutes after the administration of bicuculline (0.5 mgm/kg i.v.).

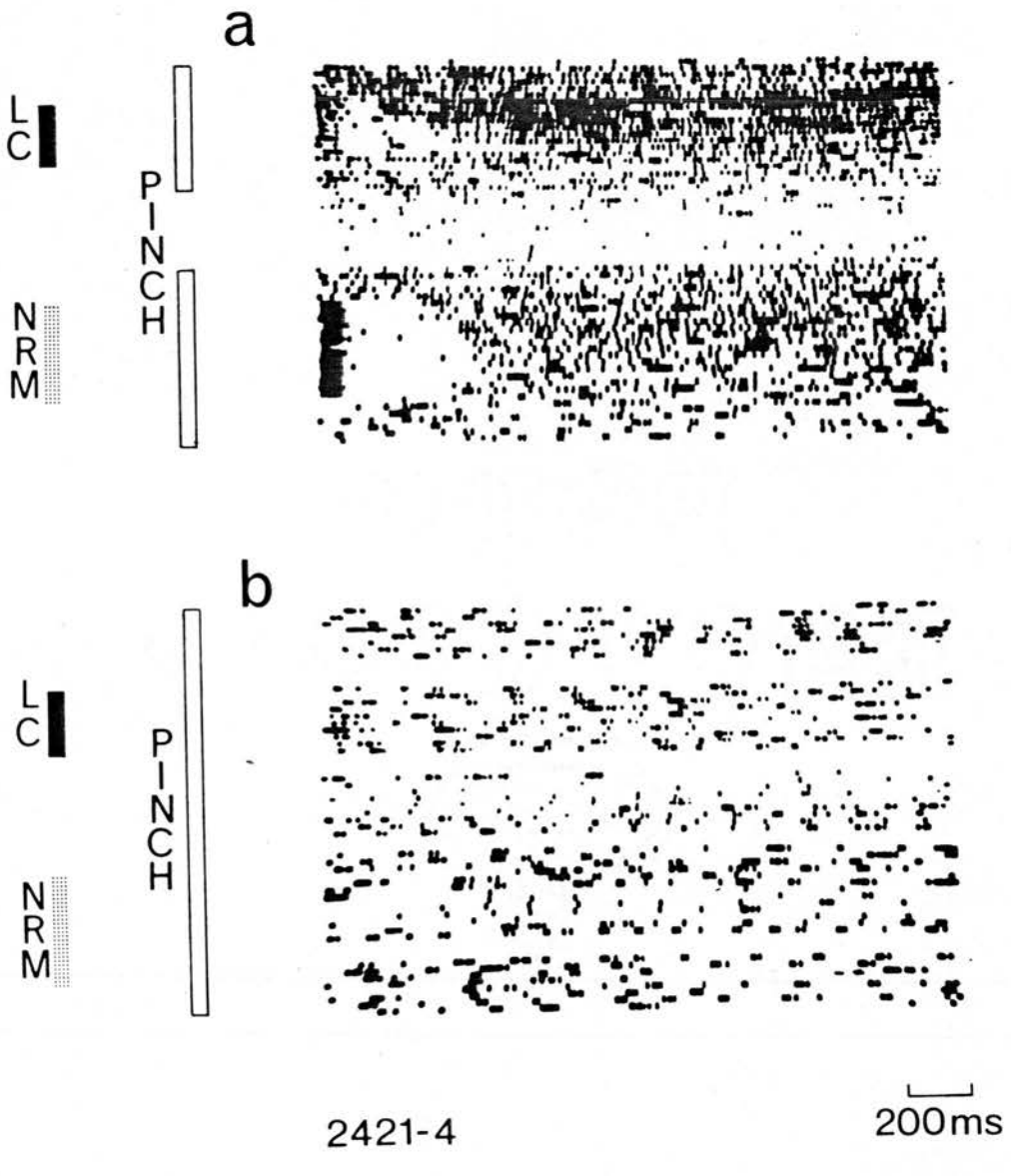

a
LC
PINCH
NRM
b
LC
PINCH
NRM
2421-4
200ms

FIGURE 4.21

Effects of drugs on the blood pressure

A - E:

The figures show the effect of methysergide (A), sotalol (B), yohymbine (C), phenoxybenzamine (PBZ) (D) and bicuculline (E) on the blood pressure. The time of intravenous application of the drugs is indicated by the arrows.

The effect of each drug was tested on a different animal.

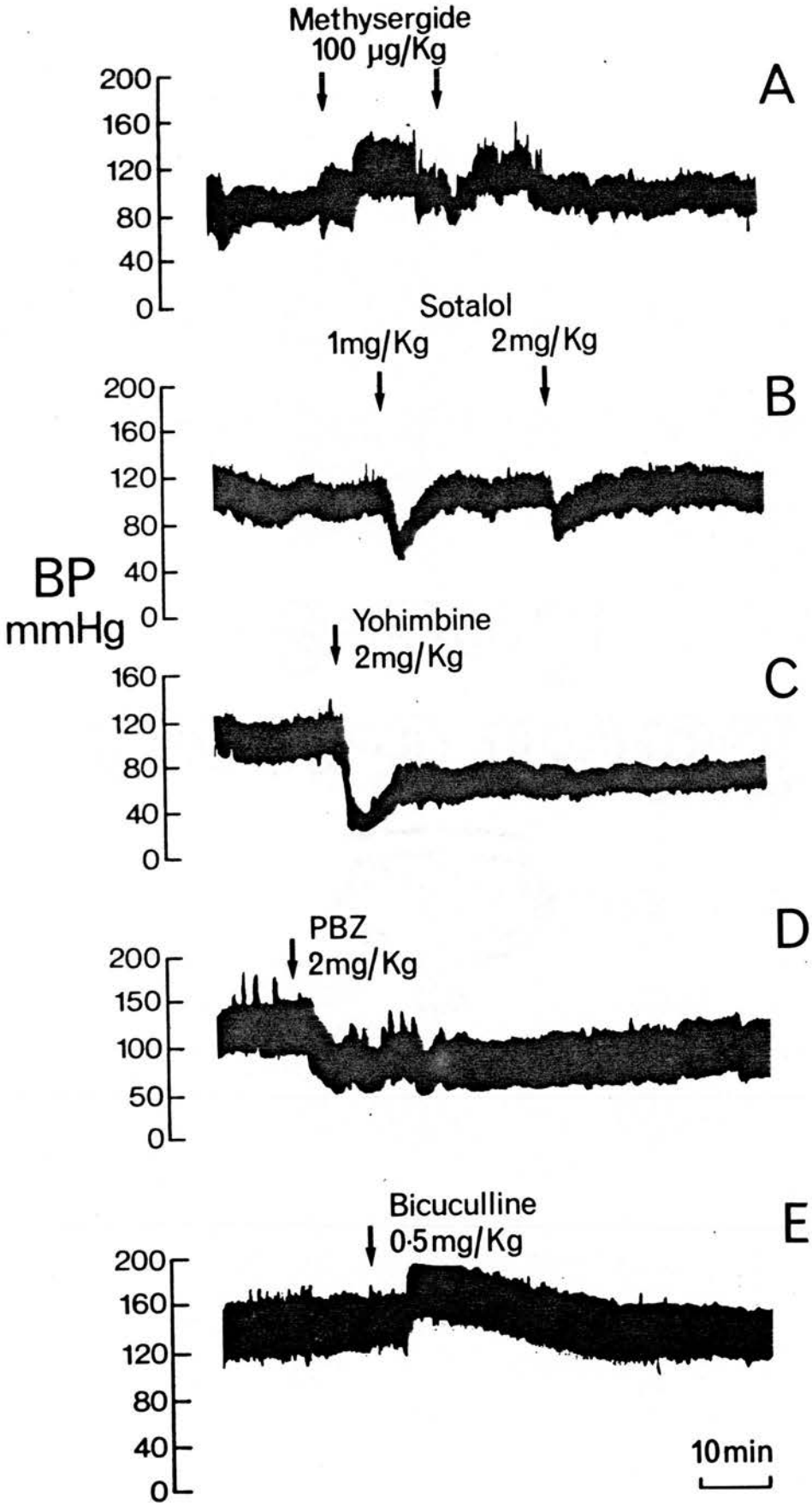

Methysergide
100 µg/Kg
A
200
160
120
80
40
0
Sotalol
1mg/Kg
2mg/Kg
B
BP
mmHg
Yohimbine
2mg/Kg
C
PBZ
2mg/Kg
D
150
100
50
Bicuculline
0·5mg/Kg
E
10min

FIGURE 4.22

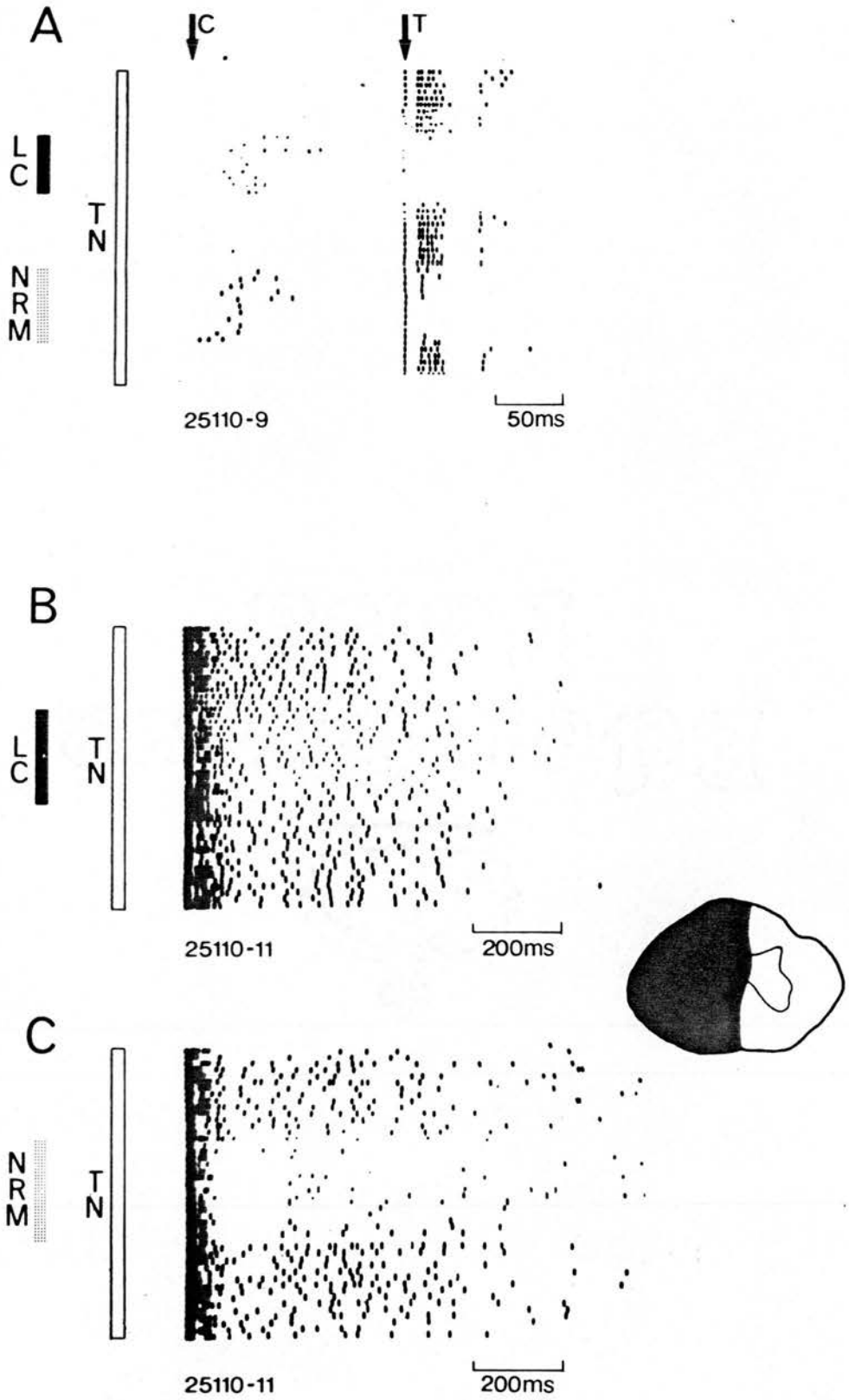
Effect of selective lesions in the spinal cord on the action produced from LC and NRM

A : Before the lesion

The raster display shows the effect of stimulation in the locus coeruleus (LC) and the raphe magnus (NRM) on the discharge evoked by the stimulation of the tibial nerve (test stimulus, 10 V, 0.5 m sec pulse width) applied 100 m sec after the application of the conditioning stimulation in the LC and NRM as indicated by arrows. A train of 60 m sec (200 HZ) at 300 μA was used to stimulate the LC and NRM. The train of stimuli was repeated once every second.

B,C : After the lesion

The effect of ipsilateral hemisection on the inhibition produced from LC and NRM on another neurone. The stimulation parameters from LC and NRM were the same as above (A). The tibial nerve was stimulated at 15 Volts (0.5 m sec pulse width). The extent of the lesion is shown by the black area in the spinal cord section on the right-hand side of the figures.


A
C
T
LC
TN
NRM
25110-9
50ms
B
LC
TN
25110-11
200ms
C
NRM
TN
25110-11
200ms

FIGURES 4.23 - 4.26

The effect of LC and NRM stimulation prior to the lesions made in the spinal cord

4.23

A,B,C, : The raster displays from three different neurones show the effect of LC and NRM stimulation on the discharge evoked by the electrical stimulation (A), by the noxious pinch (B) and on the background discharge (C) prior to making any lesions. In each case the responses to LC and NRM stimulation were similar.

The figures that follow (4.24 - 4.26) are from the same animal. The LC and NRM stimulation parameters were as follows:

300 μA
200 μ sec pulse width
60 m sec train
300 HZ
repeated 1/1200 m sec

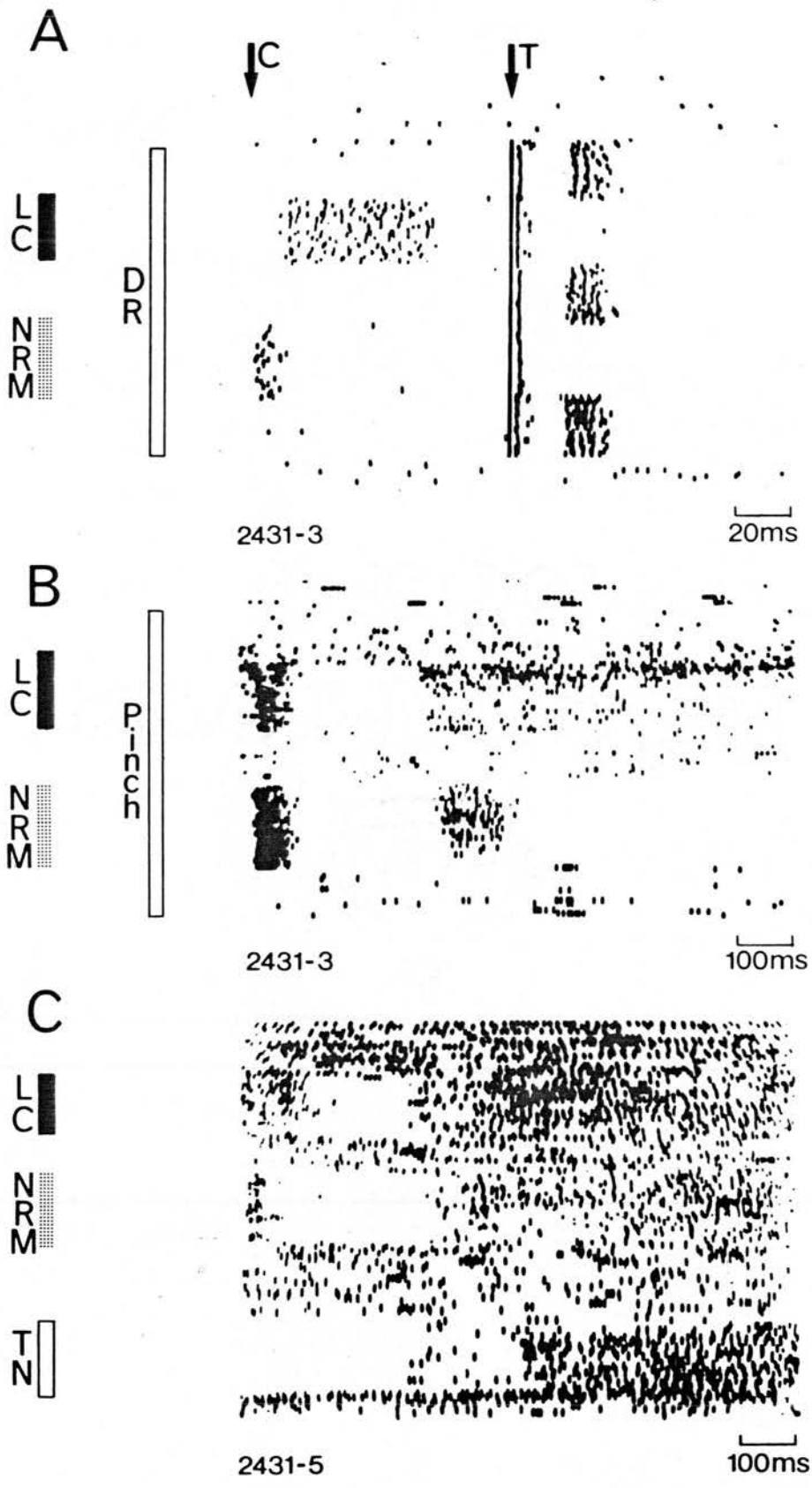

A
C
T
LC
NRM
DR
2431-3
20ms
B
LC
NRM
Pinch
2431-3
100ms
C
LC
NRM
TN
2431-5
100ms

FIGURE 4.24

A,B : The raster displays show the effect of making the lesion, shown on the right-hand side (black area) of the figures, on the effects produced from LC and NRM on two multireceptive neurones. This figure should be compared with the figure 4.23 which shows the results prior to any lesion in this animal. In each of these units the effects of NRM stimulation were much stronger than those from LC stimulation.

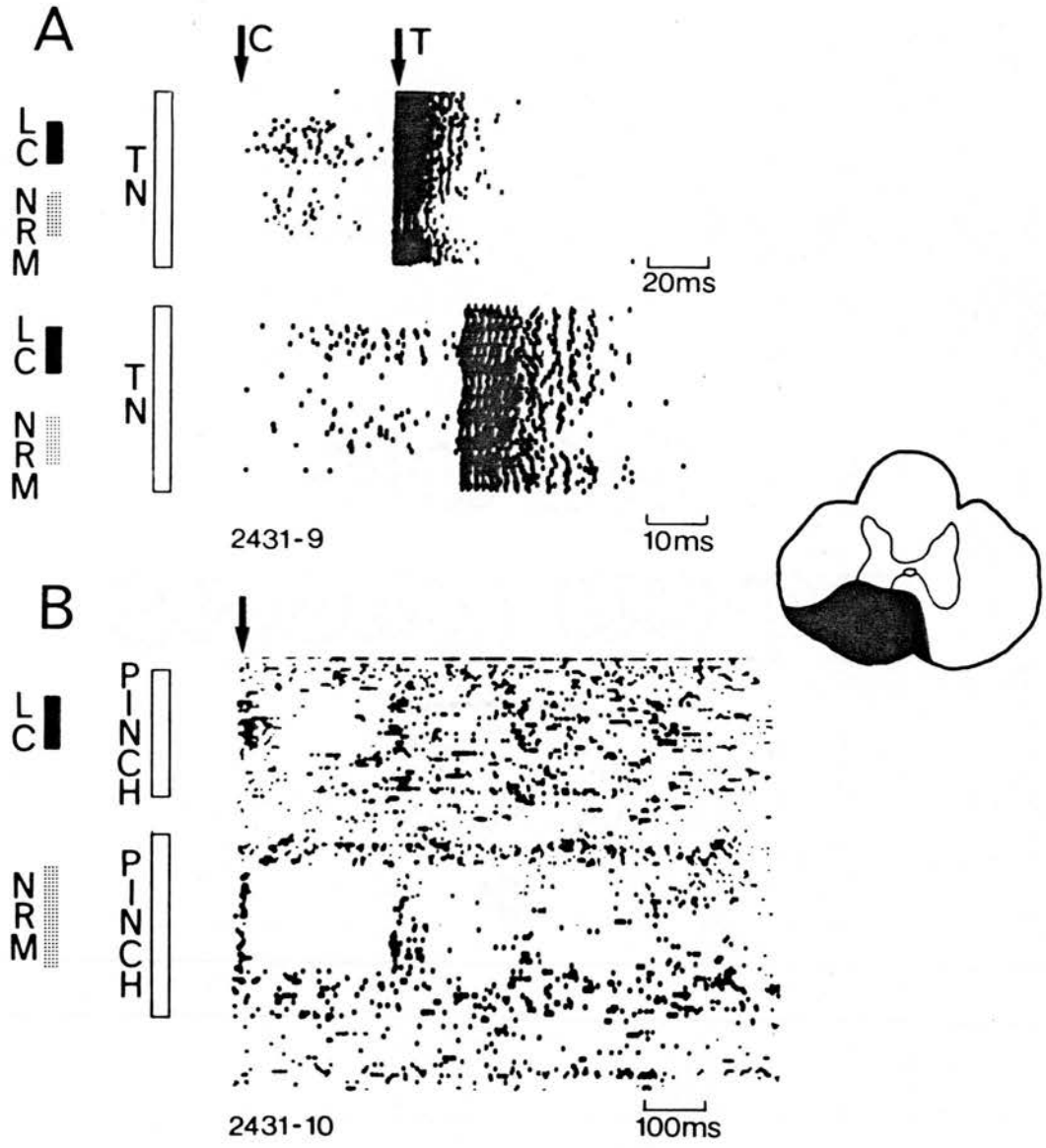

A
C
T
LC
NRM
TN
20ms
2431-9
10ms
B
PINCH
2431-10
100ms

FIGURE 4.25

a,b,c : The raster display of a single neurone held after several progressive lesions were made in the spinal cord, as shown on the right-hand side of the figures. The blackened area in the spinal cord sections shows the current lesion prior to the results in the raster display and the shaded areas show the previous lesions made in the same animal. The unit showed wind-up to the stimulation of the tibial nerve at 20 Volts (0.5 m sec pulse width) repeated every 1.2 seconds.

This unit was first encountered after the ipsilateral VLF had been sectioned (a) and stimulation in LC had no demonstrable effect on the response to tibial stimulation whereas NRM stimulation strongly inhibited this response. The effect from NRM was greatly reduced after the ipsilateral DLF was lesioned as well (b). The effect produced by stimulation in NRM was completely abolished on making a subsequent contralateral DLF lesion (C).

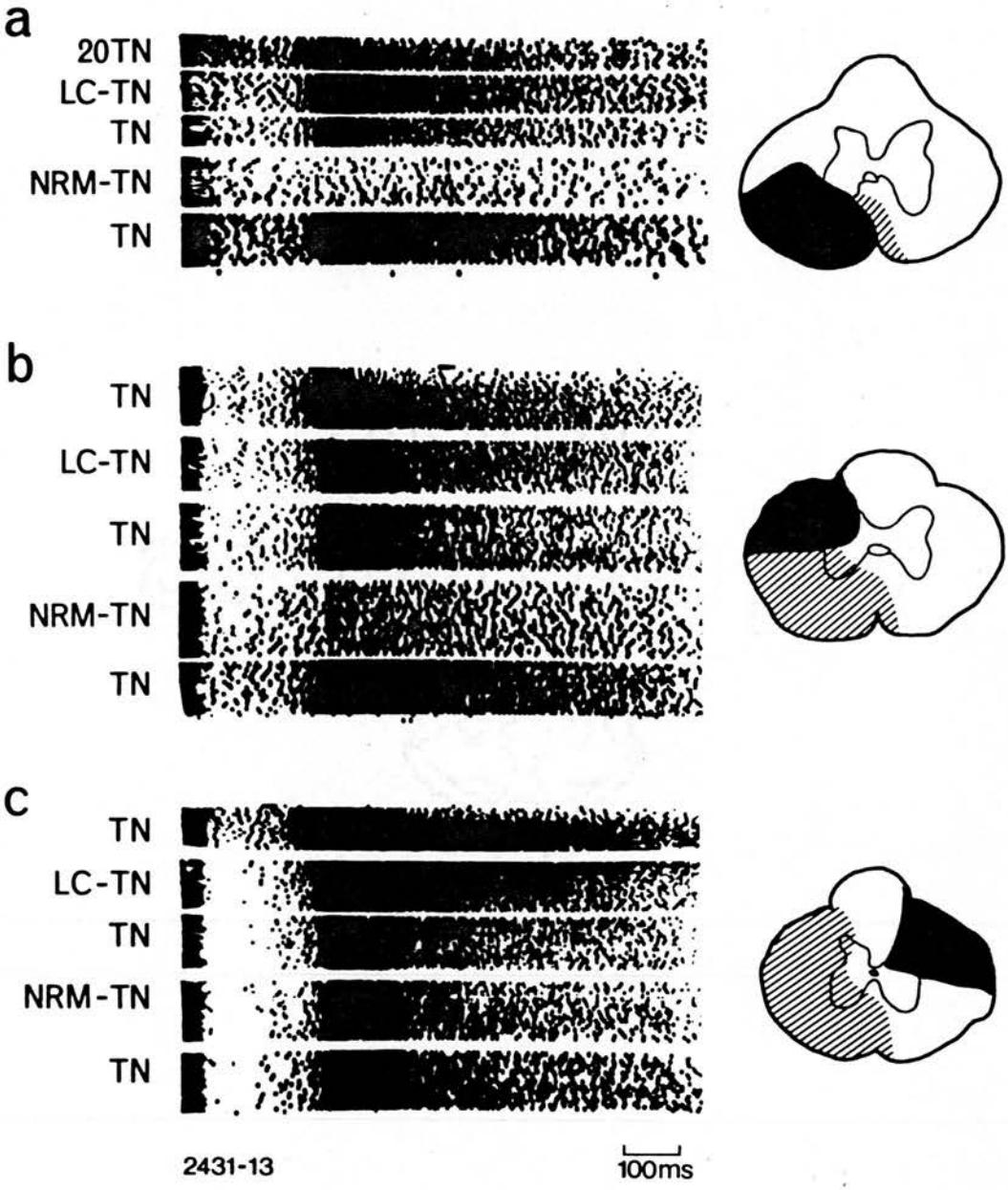

a
20TN
LC-TN
TN
NRM-TN
TN
b
TN
LC-TN
TN
NRM-TN
TN
c
TN
LC-TN
TN
NRM-TN
TN
2431-13
100ms

FIGURE 4.26

a : The figure shows the spinal cord location of the recording site for the unit in Fig. 4.25 c.

b,c : The stimulation sites in the LC and NRM for the experiment described in figures 4.23 - 4.25. The filled circle and triangle illustrate the sites of stimulation.

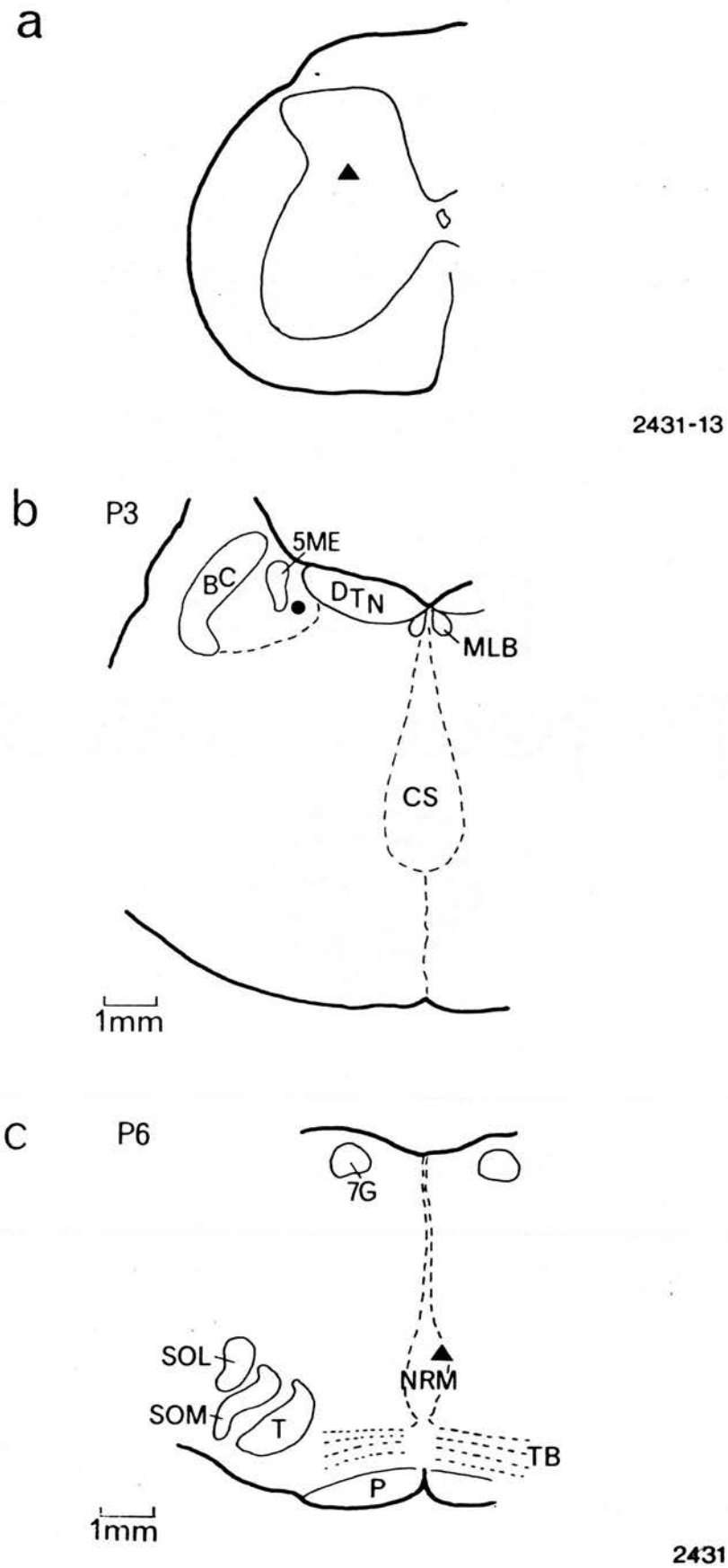
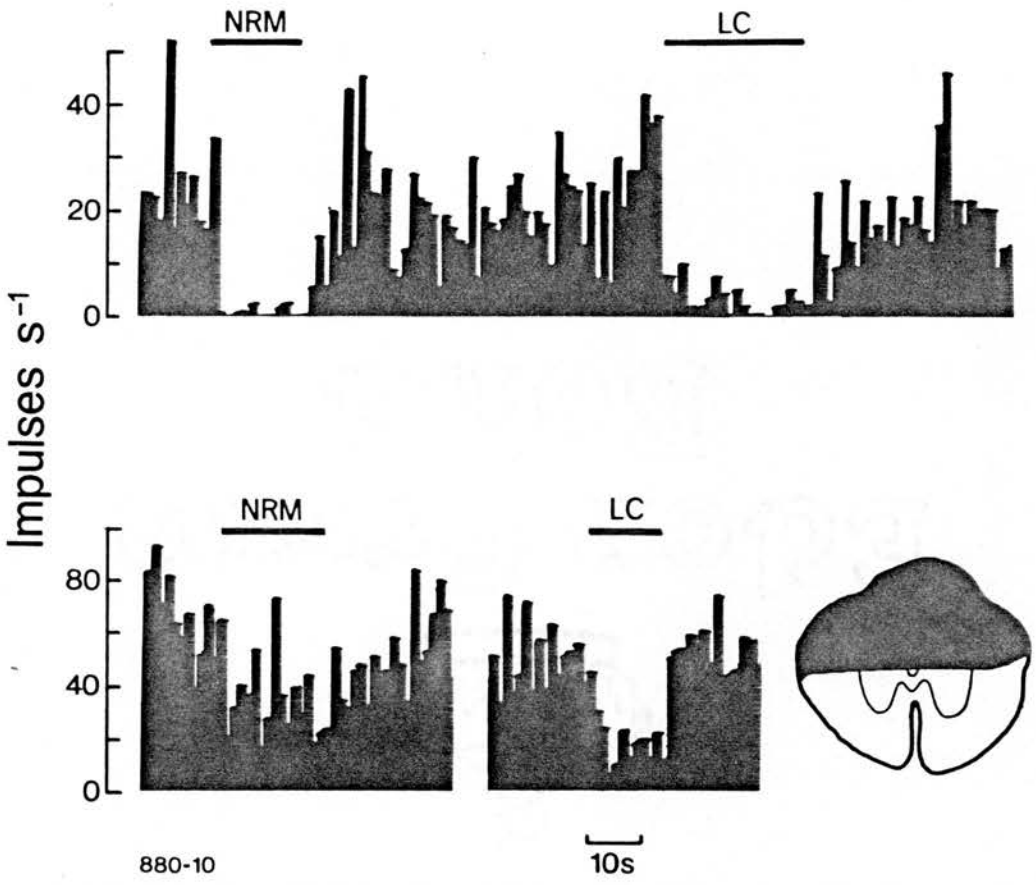

a
2431-13
b
P3
5ME
BC
DTN
MLB
CS
1mm
c
P6
7G
SOL
SOM
T
NRM
TB
P
1mm
2431

FIGURE 4.27

Effect of a lesion involving bilateral DLF on the inhibition from LC and NRM

The top frequency histograms illustrate the background discharge of this multireceptive neurone and the effects of LC and NRM stimulation when the spinal cord was intact. After making the spinal cord lesion shown on the right-hand side of the bottom part of the figure, effects of identical stimuli applied in the LC and NRM are shown. The response to LC stimulation is still strong after the lesion whereas the NRM stimulation evoked inhibition is greatly reduced.


NRM
LC
40
20
0
Impulses s-1
NRM
LC
80
40
0
880-10
10s

FIGURES 4.28 - 4.29

Effect of making lesions in the midline raphe complex on the inhibition from LC

A,B : Frequency histograms illustrating the background discharge of two different neurones and the effect of LC and NRM stimulation prior to making the midline raphe lesions shown in figure 4.29.

In Figure B the train length of LC stimulation was changed from 80 m sec to 60 m sec in the second trial.

C : The effect of making midline raphe lesions shown in figure 4.29 on the inhibition from LC. The inhibitory effect produced by stimulation in LC was still intact after the lesions were made as shown in figure 4.29.

D : The site of stimulation in the LC in this experiment.

NRM and LC were stimulated with 30 m sec trains (300 μA, 200 μsec, 500 HZ) repeated thrice every second (Fig. A). Similar parameters were used in B excepting the train length. Similar parameters were used in C excepting the train length which was 60 m sec and 80 m sec to stimulate LC and NRM respectively.

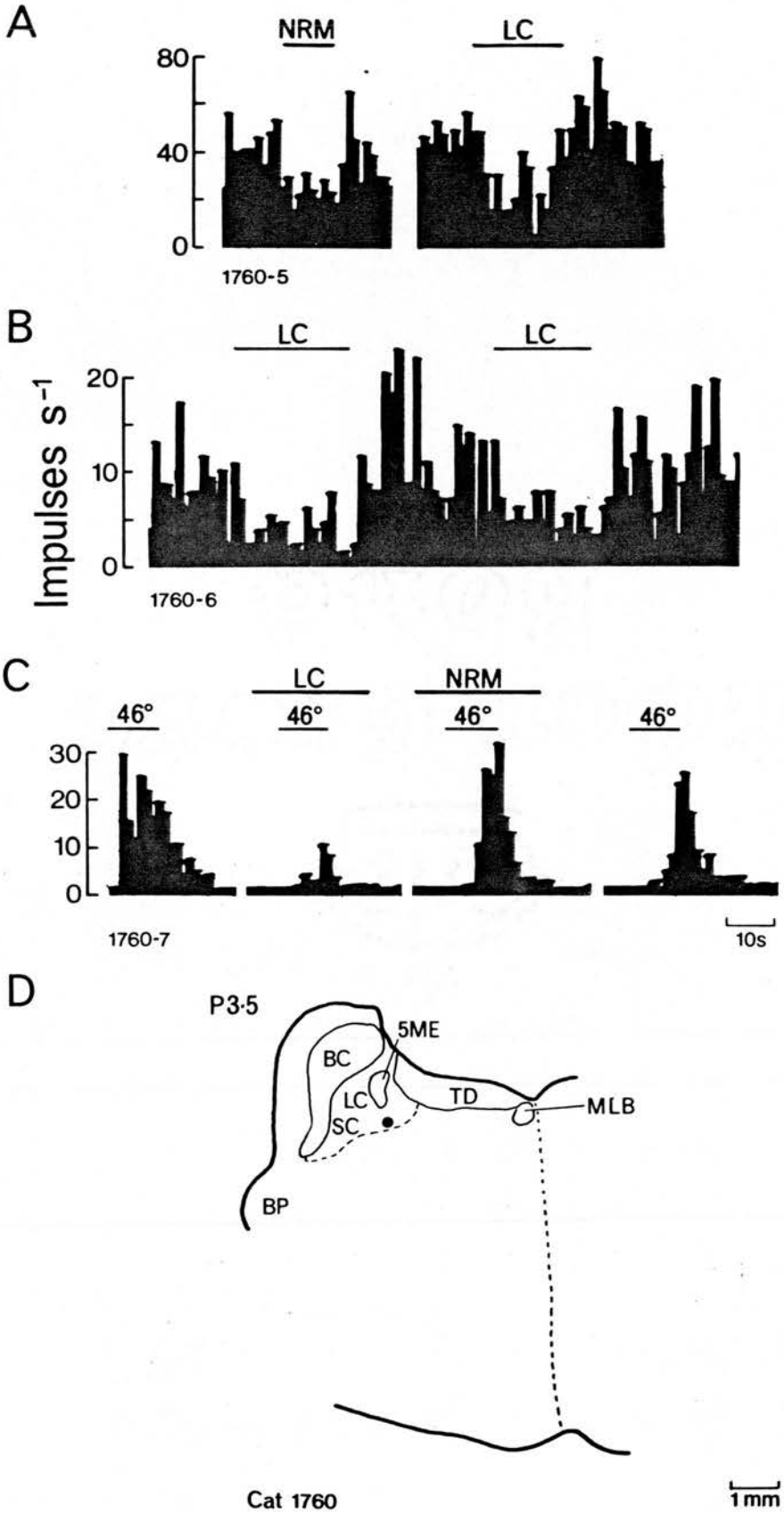

A
NRM
LC
80
40
0
1760-5
B
LC
LC
Impulses s-1
20
10
0
1760-6
C
LC
NRM
46°
46°
46°
46°
30
20
10
0
1760-7
10s
D
P3·5
5ME
BC
LC
SC
TD
MLB
BP
Cat 1760
1mm

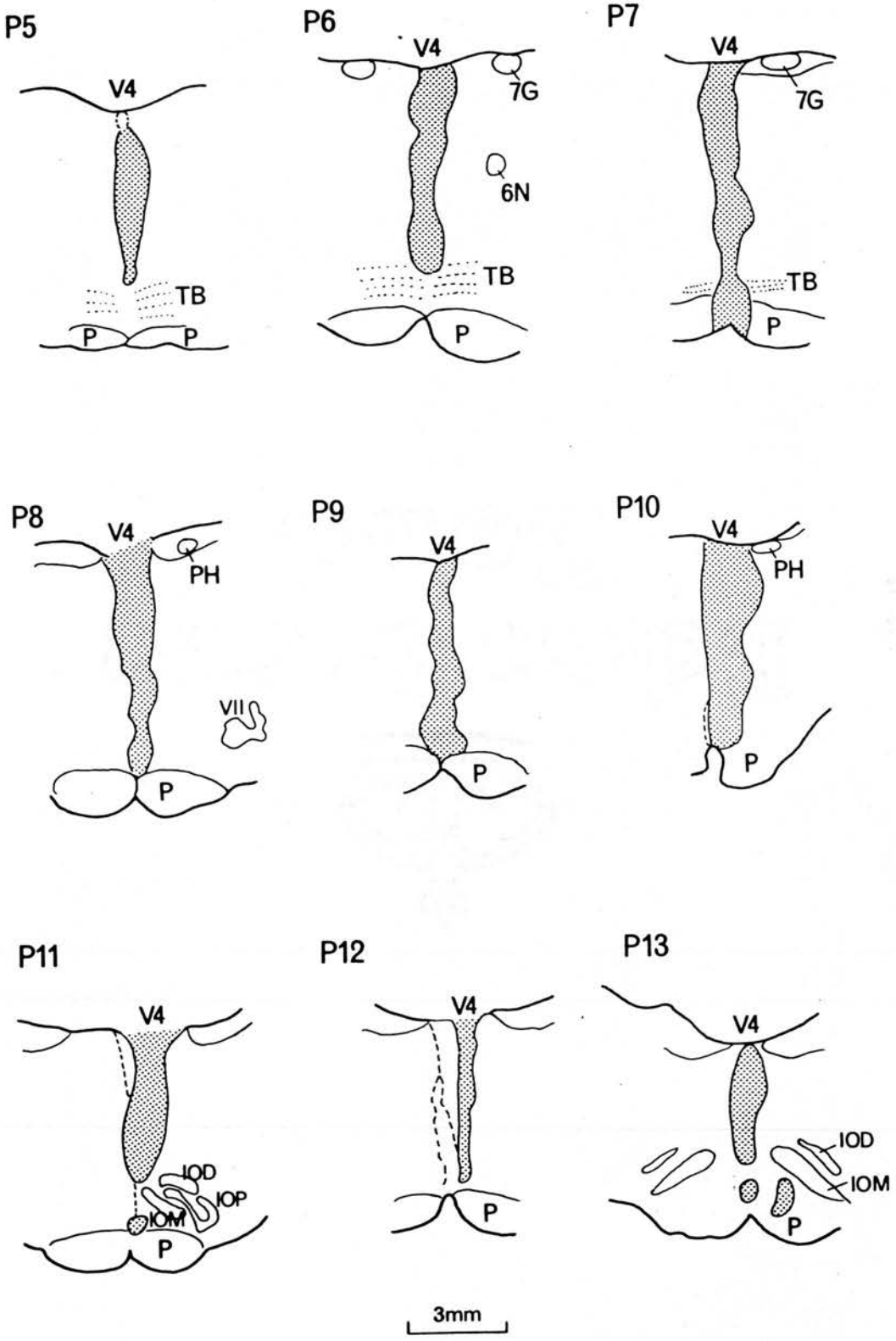
FIGURE 1.29

The extent of midline raphe lesions

The figures show the extent of electrolytic lesions made in the region of the midline raphe complex. The stippled areas enclosed by solid lines show the extent of the lesion and the broken lines represent the midline area that was not completely lesioned.

Abbreviations:

6N	:	abducens nucleus
7G	:	genu of the facial nerve
VII	:	facial nucleus
IOD	:	inferior olive, dorsalis
IOM	:	inferior olive, medialis
IOP	:	inferior olive, principalis
P	:	pyramidal tract
PH	:	nucleus praepositus hypoglossi
TB	:	trapezoid body


P5
V4
TB
P
P
P6
V4
7G
6N
TB
P
P7
V4
7G
TB
P
P8
V4
PH
VII
P
P9
V4
P
P10
V4
PH
P
P11
V4
IOD
IOP
IOM
P
P12
V4
P
P13
V4
IOD
IOM
P
3mm

1760

FIGURE 4.30

Effects of selective spinal cord lesions on DRPs evoked by stimulation of LC and NRM

a : After making the lesion shown, there was no change in the DRPs evoked from LC and NRM.

b : After making the second lesion shown by the blackened area, the DRP evoked by NRM stimulation disappeared as shown in c. The shaded area shows the lesion made in (a).

c : The evoked DRPs from the tibial nerve (TN), LC and NRM after making the lesions shown in a and b. The recordings are contaminated by 50 HZ interference, but the potentials recorded were typical DRPs. The DRP evoked from NRM was abolished after the lesions (a, b) whereas the LC evoked DRP was still intact.

d : After making the fourth lesion (blackened area) the DRP from LC was also abolished. The shaded areas represent the previous lesions made in a and b.

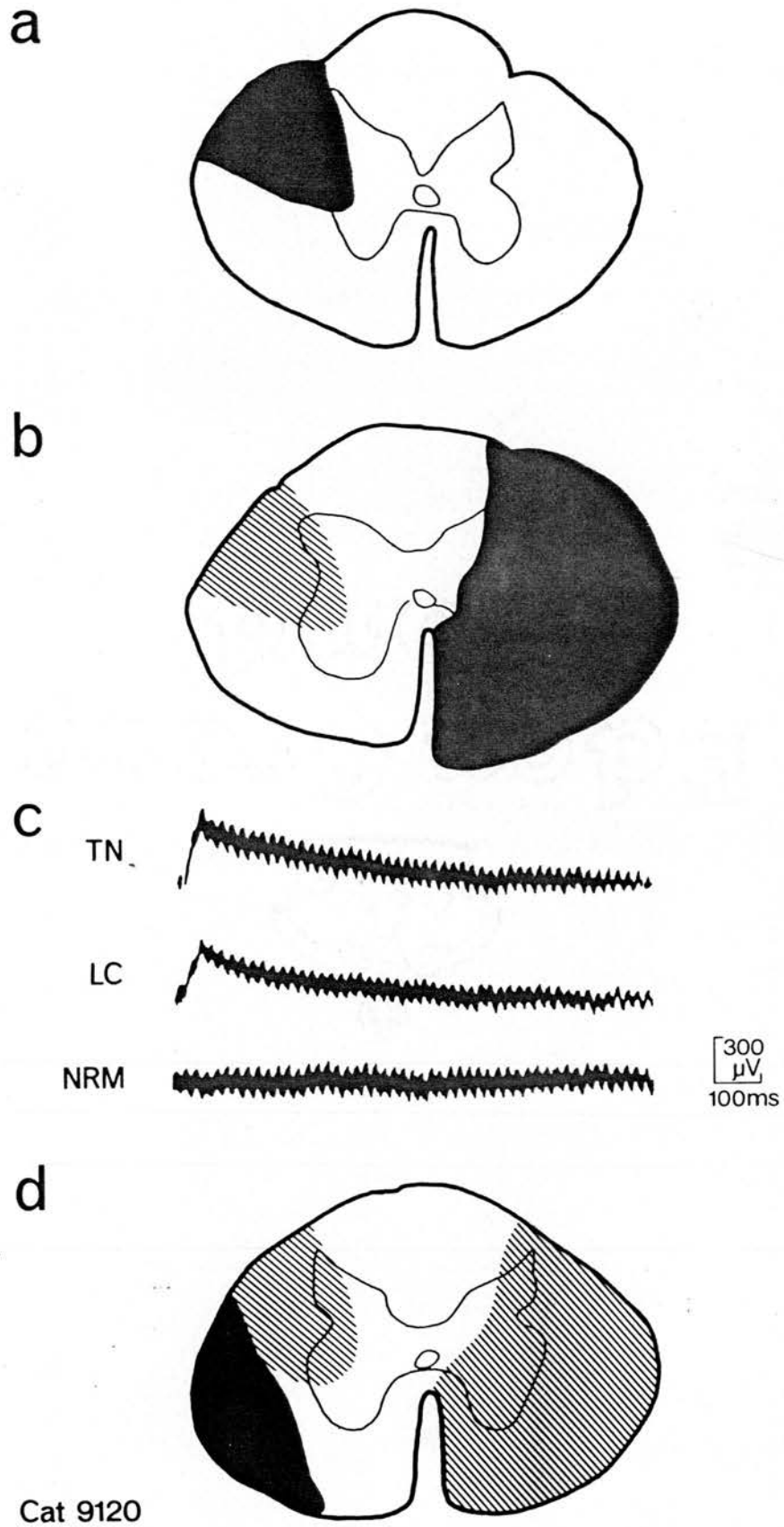

a
b
c
TN
LC
NRM
300
μV
100ms
d
Cat 9120

FIGURE 4.31

Effects of selective lesions in the spinal cord on DRPs evoked from LC and NRM

a - e : The figures show the effect of making various lesions on the DRPs evoked from the LC and NRM. Each record in b - d was taken after making the lesion shown in the right-hand side by the blackened area in the spinal cord section. The shaded areas represent the lesions made previously in the same animal.

After making the lesion shown in b, DRPs evoked by threshold stimulation (a) were abolished and higher stimulus intensities were required to evoke DRPs.

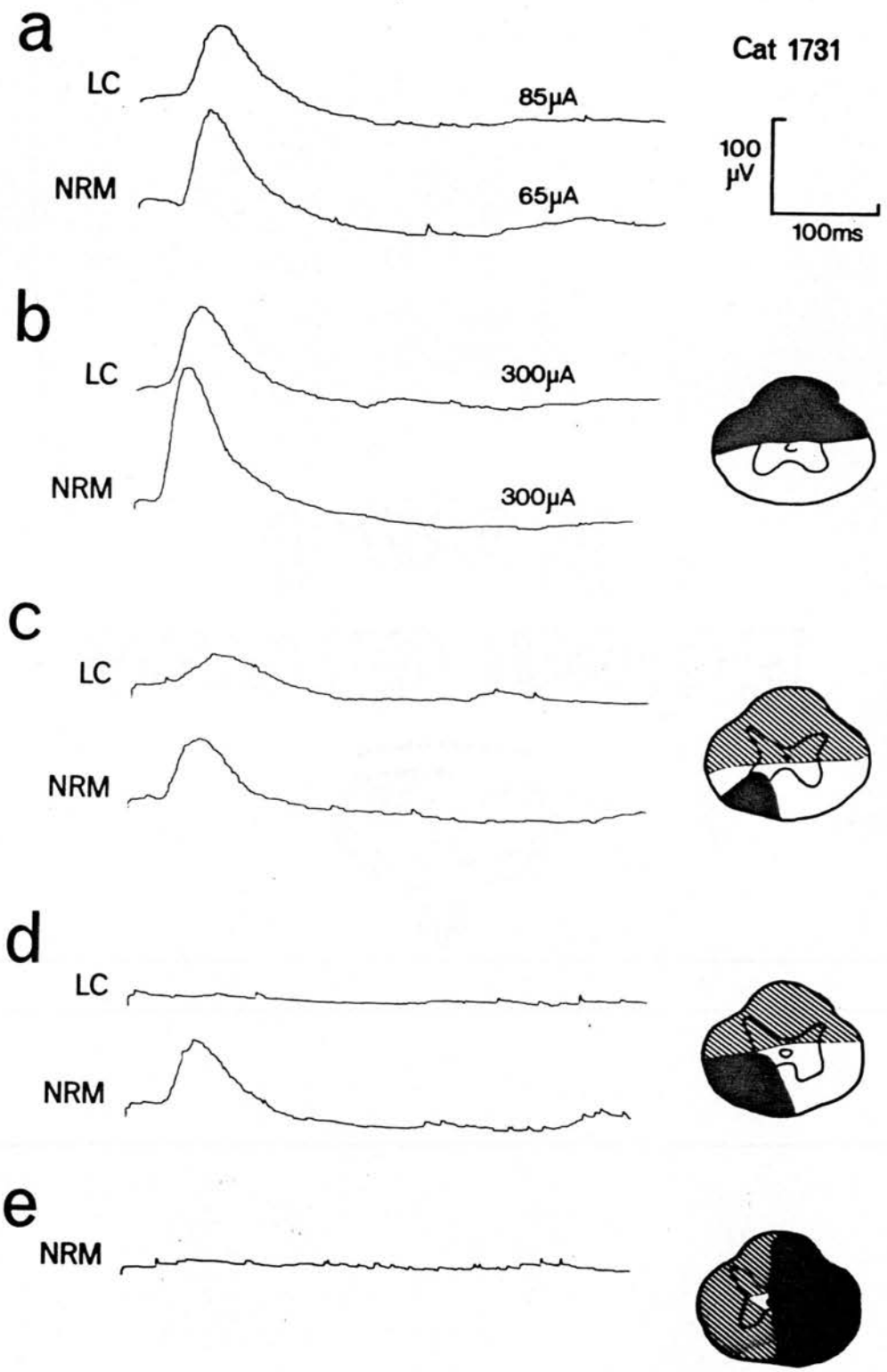

a
LC
NRM
85μA
65μA
Cat 1731
100
μV
100ms
b
LC
NRM
300μA
300μA
c
LC
NRM
d
LC
NRM
e
NRM

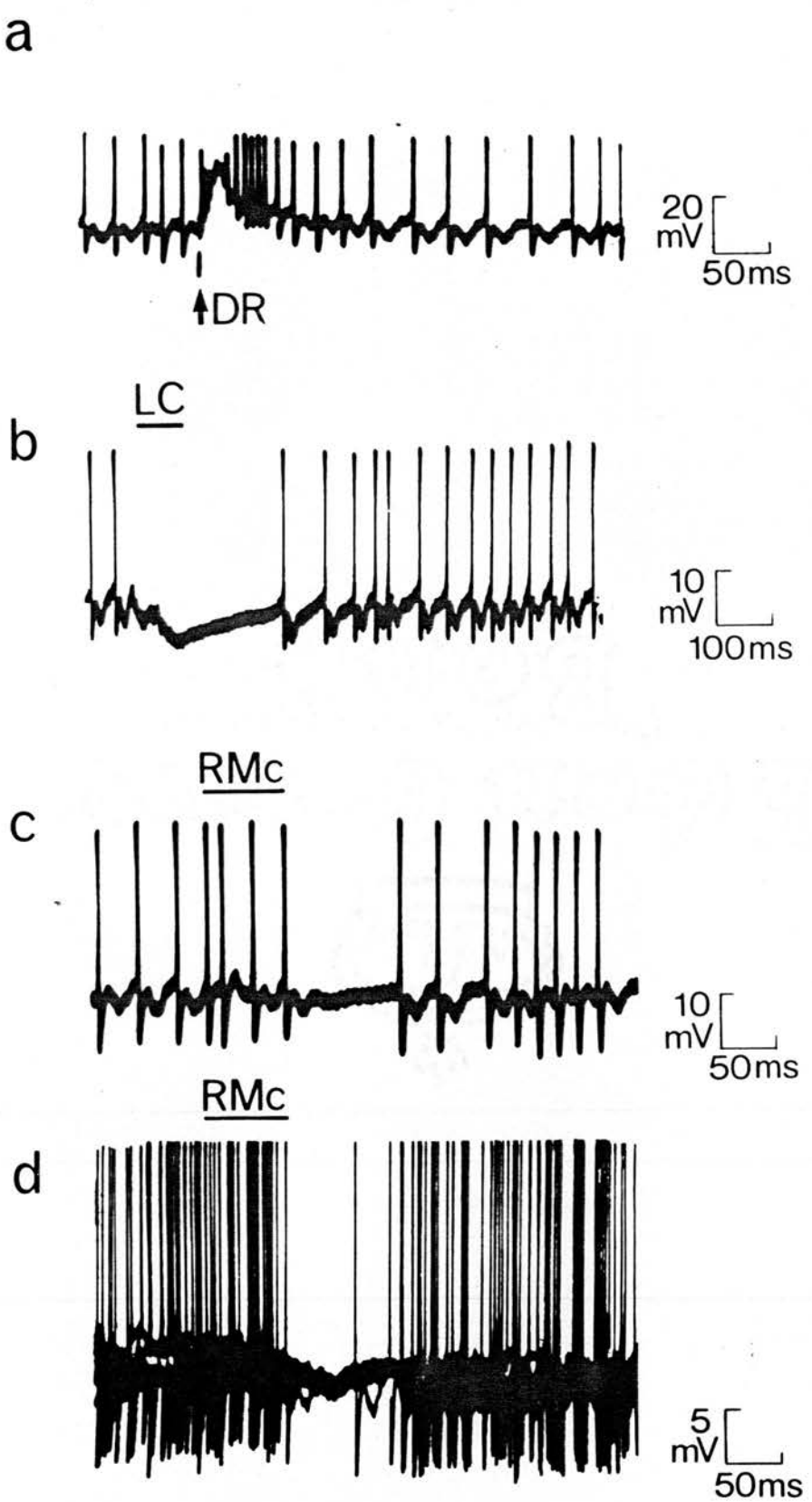
FIGURE 1.32

Intracellular recording

a - d : This is d.c. intracellular recording from multireceptive neurone and shows the EPSP caused by the dorsal root stimulation (a) and the IPSPs produced by the stimulation in LC (b) and the nucleus reticularis magnocellularis (c, d). The bars on top of figures (b - d) represent the duration of the train of stimuli applied in the LC and RMc. A train of stimuli indicated by the bars was repeated once every 1500 m sec. The unit was spontaneously active. *

e,f : The figures show the sites of stimulation in the LC and RMc that evoked the IPSPs shown in b - d. The filled circles represent the sites of stimulation.

* Records in a-c are from single sweeps whereas five superimposed traces are shown in d. A 80 m sec long train (500 HZ, 200 μsec pulse width) at 300 μA was used to stimulate LC and RMc.


a
20
mV
50ms
DR
b
LC
10
mV
100ms
c
RMc
10
mV
50ms
d
RMc
5
mV
50ms
2120-2

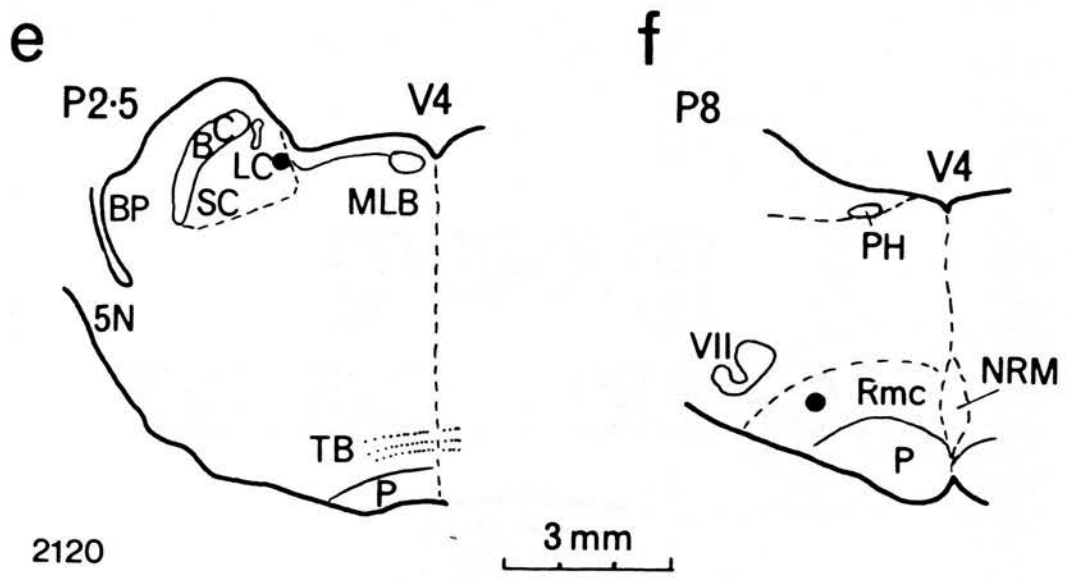

e
P2·5
V4
BC
LC
BP
SC
MLB
5N
TB
P
2120
3 mm
f
P8
V4
PH
VII
Rmc
NRM
P

FIGURE 4.33

Effect of bicuculline on the evoked DRPs

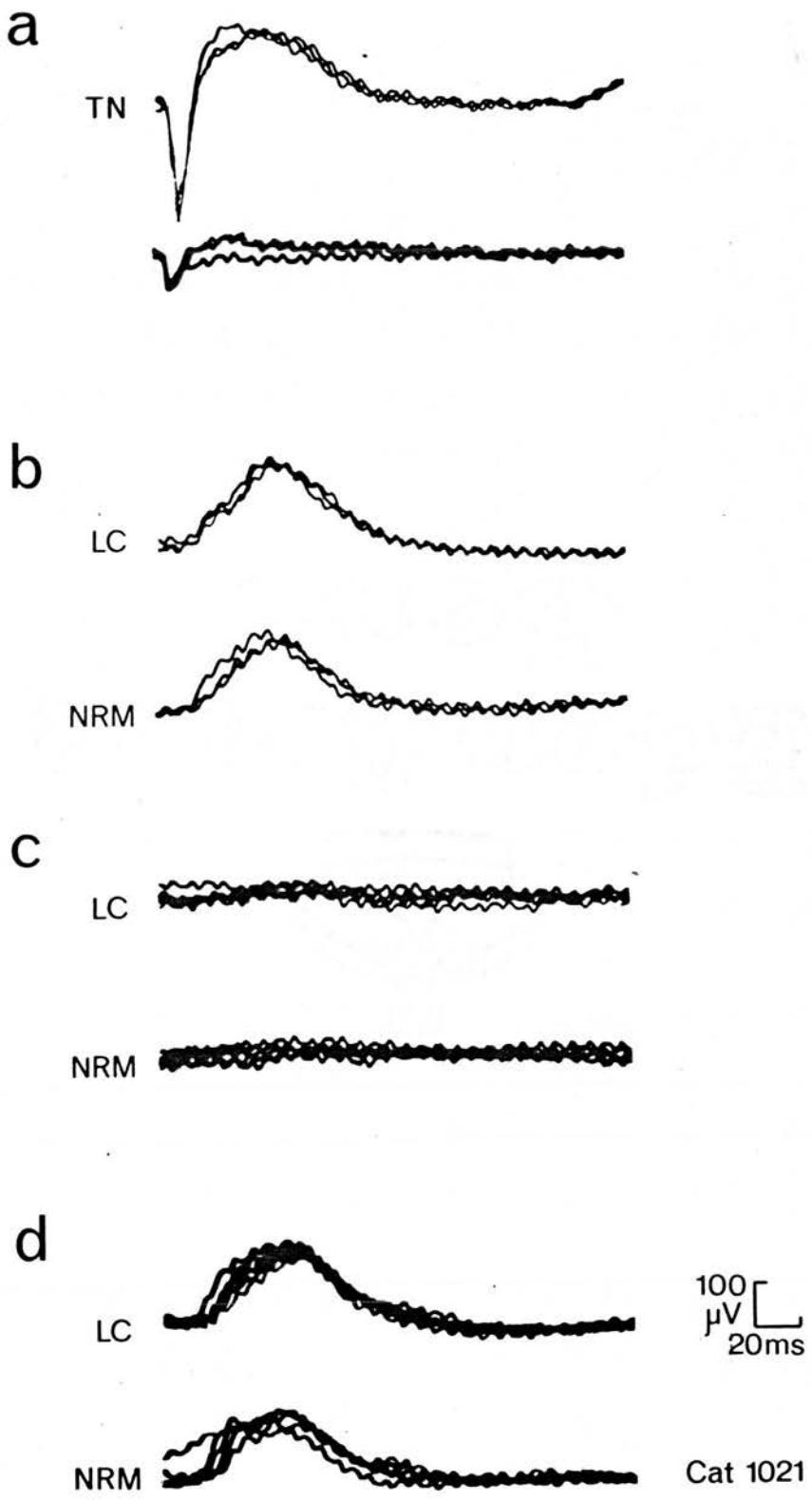
a : DRPs evoked by the stimulation of the tibial nerve before (top) and after bicuculline 0.5 mgm/kg given intravenously.

b : Control DRPs evoked from LC and NRM before the administration of bicuculline.

c : Bicuculline (0.5 mgm/kg) abolished the DRPs from LC and NRM as shown and also abolished the DRP evoked by the tibial nerve (a, bottom part).

d : Reappearance of evoked DRPs within 70 minutes of bicuculline administration.

Time constant of 1 second was used for DRP recordings.


a
TN
b
LC
NRM
c
LC
NRM
d
LC
NRM
100
μV
20ms
Cat 1021

SECTION V

GENERAL DISCUSSION AND CONCLUSIONS

The present investigation was carried out on spinal cord neurones responding to noxious and non-noxious stimulation. The projection of these neurones into the spinothalamic tract, and various segmental and supraspinal influences exerted on these neurones were investigated. The evidence provided will enhance the understanding of the neural mechanisms underlying the perception of pain.

The multireceptive neurones examined in the present investigation were found to be distributed all over the dorsal horn including lamina I. The presence of specific nociceptor-driven neurones has been demonstrated in lamina I (Kumazawa et al. 1975; Cervero et al. 1976). It has also been shown recently by intracellular staining of identified primary afferents that lamina I and dorsal part of SG receive direct projection from cutaneous nociceptors whereas they do not receive a direct projection from non-nociceptors (Light and Perl, 1979 b). The finding of the present investigation showing the existence of multireceptive neurones in lamina I becomes paradoxical in view of the above-mentioned evidence. However it has been suggested that stalked-cells in SG which can receive non-noxious excitatory monosynaptic input from $A\delta$ fibres can relay this information on to lamina I neurones (Price et al. 1979). There is anatomical evidence that suggests that $A\delta$ fibres from D-hair do have terminations in the innermost part of SG (Light and Perl, 1979 b) and intracellularly stained stalked cells with dendritic arbors in the outer SG have been reported to respond to noxious and non-noxious, or noxious cutaneous stimulation alone (Abdelmoumene et al. (1981). The axons of these neurones arborize extensively in

lamina I (Price et al. 1979). The observations of Price et al. (1979) that neurones within a pair (recorded in laminae I and II) often received convergent input from the same primary afferents and lamina II neurones displayed shorter latency than lamina I neurones, does suggest the role of some SG interneurones acting as excitatory interneurones relaying information to lamina I. These findings explain the observation of multireceptive neurones in lamina I in the present investigation. The role of these neurones in nociceptive transmission and how their activity can be modulated from segmental and supraspinal levels will be discussed further.

The spinothalamic tract has been a classical pain pathway for over a century and the idea was mainly based on the observation of the loss of pain sensation contralateral and caudal to a ventrolateral quadrant lesion. The spinothalamic tract in the primate has been well studied and it was revealed that a substantial proportion of its composition contains multireceptive neurones (Willis et al. 1974; Price et al. 1978). Some of the spinothalamic neurones in the primate respond in a manner analogous to human pain sensation evoked by the same stimuli. Although the spinothalamic pathway in the cat is well defined anatomically, the secrets surrounding the somatosensory properties of these cells have not been unmasked so far. The present investigation revealed that the spinothalamic tract in the cat also contains a substantial proportion of its cells which are of the multireceptive type. Based on the experimental evidence provided, it is suggested that the spinothalamic tract is involved in the transmission of noxious and tactile messages to the brain which will be important for the sensory discriminative aspects

of pain perception. It is possible that the spinothalamic pathway in the cat is small compared to the well developed projection in the monkey. Two possibilities that arise from here are these: the spinothalamic tract in the cat either despite its small size plays a similar function in the somatic sensation as in the primate or else it has been replaced, up to a certain extent, by other ascending pathways such as the spinoreticular tract (Albe-Fessard et al. 1974; Fields et al. 1977 a). The spinoreticular tract neurones have been demonstrated to be concentrated mainly in laminae VII and VIII and also respond to noxious cutaneous stimulation (Fields et al. 1977 a). The large number of multireceptive neurones that projected to the contralateral VLQ but could not be demonstrated to project to the thalamic nuclei in the present study will support such a suggestion as that. However some unknown factors might also have masked a proportion of the spinothalamic tract neurones.

Nociceptive transmission in the spinal cord has been demonstrated to be under a strong modulatory influence from both the segmental and the supraspinal levels in the present investigation. The dorsal horn is known to contain enkephalins, opiate receptors and substance-P and their distributions overlap considerably in the superficial dorsal horn. The opiate receptors are presumably located on the small diameter afferents that contain substance-P which may be involved in the mediation of nociceptive transmission to the dorsal horn from the high threshold cutaneous afferents. Enkephalins are suggested to be present either in the interneurones or in the descending fibres. The evidence provided in the present investigation however does not suggest the involvement of endogenous

opioids in inhibition of multireceptive neurones, generated by stimulation of the dorsal columns, by contralateral plantar nerve or by tonically active descending pathways. The participation of opioids, GABA and 5-HT in the tonic descending inhibition has not been demonstrated in other studies as well (Duggan et al. 1980; Griersmith et al. 1981; Johnson and Duggan, 1981). However the evidence from Lundberg's laboratory (Engberg et al. 1968 b) did demonstrate that monoaminergic transmission plays a part in the tonic descending control of reflex transmission. The experiments conducted in the final part of the present investigation revealed an increased rate of discharge of multireceptive neurones on administration of β-blockers but requires further systematic examination. However the demonstration that the LC actions in the present investigation were mediated by α-receptors will perhaps suggest that β-blockers may be acting supraspinally. The failure of serotonin antagonists to antagonise the inhibition produced from NRM may reflect the lack of potent and specific antagonists for 5-HT in the spinal cord.

Nociceptive transmission has been demonstrated to be under a powerful descending inhibitory control exerted from LC. The modulation of nociceptive transmission from LC suggests the involvement of catecholamines, opioids and GABA. Although the actions produced from LC and NRM were similar on the same neurone it has been demonstrated that LC actions are not mediated through NRM and are presumably mediated directly through the coeruleospinal projections. The contributions of nuclei such as the nuclei reticularis gigantocellularis and the magnocellularis cannot be ruled out but may play only a small part. The LC actions may involve both pre- and

postsynaptic mechanisms but require further studies which are being carried out as part of a continuing long-term project.

The evidence presented shows that the spinothalamic tract in the cat containes neurones of the multireceptive type and multireceptive neurones are under a strong segmental, tonic and phasic descending (supraspinal) inhibitory control.

So far the discussion has been concerned with the nociceptive mechanisms without going into the psychological aspects which are inherent in the perception of pain. A brief and uncricitical account of the psychological dimensions of pain will be given. Three major psychological dimensions of pain, the sensory-discriminative, motivational-affective and cognitive-evaluative have been described (Melzack, 1973). These various psychological dimensions have been suggested to be subserved by different physiologically specialised systems (Melzack and Casey, 1968; Melzack, 1973; Casey, 1980). The nociceptive information can be transmitted to the brain along several ascending spinal pathways such as the spinothalamic tract, spinocervical and the spinoreticular pathways. The dorsal columns have been thought to be concerned only with touch and proprioception but recently evidence has been provided that the dorsal column postsynaptic neurones can also transmit nociceptive information to the brain (Jankowska et al. 1979; Brown and Fyffe, 1981). The slowly conducting pathways such as the spinoreticular tract with its connections to the reticular formation and the limbic system has been suggested to contribute to the motivational-affective

dimension of pain (Melzack and Casey, 1968; Casey, 1980). However the part that the spinoreticular tract might play in relaying information to the thalamus from the reticular formation should not be ignored and may be as important for the sensory information processing in the thalamus and cortex as information transmitted along other sensory pathways such as the spinothalamic and spinocervical. The spinothalamic tract in the primate has been demonstrated to send collaterals to the medial brain-stem (Price et al. 1978). It appears that the spinoreticular tract can relay sensory transmission to the thalamus and cortex from its relay in the reticular formation and the spinothalamic pathway can relay information to the reticular formation through collaterals of ascending STT axons that terminate in various thalamic nuclei and from these nuclei the information is transmitted on to the somatosensory cortex. The reciprocal connections that exist between the limbic system and the reticular formation will be of particular importance. The cognitive evaluative dimension has been suggested to be performed by the fast conducting pathways (dorsal columns) which can activate the neocortical areas (Melzack and Wall, 1965; Melzack, 1973). The neocortical activation can exert a control over the sensory-information processing in the thalamus and the sensory cortex and also on the motivational-affective mechanisms in the limbic system. The prefrontal cortex receiving intracortical fibres from the sensory and association cortex and with its projections to the limbic and the reticular formation can play an important part in the interactions between various dimensions of pain.

The evidence provided in this and several other investigations shows that the spinothalamic tract carries the nociceptive transmission to the thalamus from where it is presumably relayed to the sensory cortex. The somatosensory information, in particular, the nociceptive transmission can not only reach the thalamus carried by the spinothalamic but also reach the brain-stem structures through the collaterals of the ascending axons which could then activate the appropriate descending pathways to the spinal cord. The nociceptive transmission along the spinoreticular tract to the reticular-formation and the limbic system together with the activation of the neocortical areas by activity transmitted along the fast conducting pathway presumably leads to complex interactions between these various areas.

The various interactions described above occurring after a noxious stimulus, possibly lead to the perception of pain and the activation of neural mechanisms, perhaps hormonal mechanisms as well, which can suppress the nociceptive transmission at several levels of the neuraxis.

SECTION VI

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APPENDIX

Some of the work reported in this thesis has been published in the following journals:

Neuroscience Abs.	(1978) 4, 554
J. Physiology	(1978) 285, 58P
Brain Research	(1980) 182, 186-190
Q.J. Exp. Physiol.	(1980) 65, 181-188
Proc. I.U.P.S., Budapest,	(1980) Vol. XIV, P.590

a) This thesis is entirely composed by me.

b) The experiments described in sections II and III were done jointly with Dr. J.A. Holloway, Dr. R.E. Fox and Dr. J.G. Sinclair but a substantial contribution was made by me to their planning, execution, analysis and interpretation. The project described in Section IV was independently developed by me and Dr. J.A. McMillan participated in some of the experiments at a later stage of the project.